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From Medical Department C (Head P From Hansen M D) Copenhagen County Hospital in Glostrup and Medical Department B (Head P Riis, M D), Copenhagen County Hospital in Gentofte Denmark

Clinical Assessment of the Peroral Metopirone Test

By

JØRN DITZEL

Chart et al (4) synthesized in 1958 the compound 2 methyl 1, 2 bis (3 pyridyl) 1 propanone also named SU 4885 metyrapone or Metopirone® The specific effect of this compound consists of an inhibition of the 11 β oxidation process in the adrenal cortex so that the biosynthesis of certain corticosteroids especially cortisol corticosterone and aldosterone remains incomplete During the inhibition with metopirone the concentration of cortisol in the blood decreases and through this fall the pituitary gland becomes stimulated to release more corticotrophin (ACTH) As long as the inhibition lasts the increased ACTH secretion will cause the production of large amounts of steroid precursors in the adrenal cortex especially 11 desoxycortisol (Reichstein s Compound S) these substances unlike cortisol are not able to inhibit the secretion of ACTH from the pituitary Compound S is therefore accumulated in the circulating blood and is excreted in the urine as tetrahydro 11 desoxycortisol (THS) The increase in the concentration of these substances can be assessed by analyses on blood for 11-desoxycortisol and 17 hydroxycorticoids or on the urine for 17 ketogenic steroids, 17 hydroxycorticoids and THS whereby an indirect expression of the function of the anterior lobe of the pituitary is obtained A definite increase in the amount of Compound S, 11 desoxycorticosterone and other precursors does not occur in pituitary insufficiency A negative metopirone test can however also be due to an insufficiency of the adrenal cortex, and a negative metopirone test must therefore be followed by an ACTH test to exclude a functional disturbance of the adrenal cortex

Many different procedures for the metopirone test have been advocated, both peroral and intravenous administration of widely varying doses over widely varying periods of time have been suggested (3 8 13 14 17)

In this investigation the metopirone test has been performed as described by

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Liddle et al (13) in a number of patients and healthy individuals, in an attempt to assess the value of this test in the diagnosis of disturbance in adrenal or pituitary function

Methods and material

The metopirone test is carried out with peroral administration of 500 mg free metopirone (Ciba Ltd) every 4 hours for 24 hours commencing at 8 a m The urinary excretion of 17 ketogenic steroids (17-KGS) in 24-hour periods was determined for 2 days prior to during and for the 2 days after the administration of metopirone, by Jorgensen s method (10) at the Hormone Department of Statens Seruminstitut Copenhagen The cases, in which only small initial amounts of excreted 17-KGS could be expected such as in hypophysectomized patients the concentration of 11-desoxycortisol (Compound S) in plasma was also determined at 8 a m before and after the administration of metopirone The analyses for plasma Compound S were carried out by Medicinsk Laboratorium' Copenhagen, by the specific isotope dilution principle as modified by Bojesen (2) and by the double tracer technique of Keston et al (12) The metopirone test was *carried out in 10 healthy individuals (4 men* and 6 women varying in age from 18 to 45 years) in 2 patients without endocrine disease and in 24 patients with confirmed or suspected disturbances of the pituitary adrenal function (Hypophysectomized (6) chromophobe pituitary adenoma (2) bilaterally adrenalectomized (2) Cushing s syndrome (3) diabetes mellitus after acidosis (2), patients during or after corticosteroid /ACTH therapy (9)) The series of normal individuals has been investigated in co operation with Mogens Jersild M D Head of Hvidore Hospital and the Hormone Department Statens Seruminstitut Copenhagen (Heads Chr Hamburger M D and Svend G Johnsen M D) None of those examined showed any sign of malabsorption or of liver or kidney insufficiency None were undergoing treatment with psychopharmaca such as chlorpromazine or meprobamate, which are known to reduce the sensitivity to metopirone (8) In selected patients an adrenocortical stimulation test was carried out by giving 50 I U ACTH in an intravenous infusion over exactly 8 hours and determining the urinary excretion of 17 KGS in the 24 hours prior to and the 24 hours which included the ACTH infusion

Results

The results of all metopirone tests are listed in tables I—II

Normal individuals

In all 10 healthy individuals a marked increase of the urinary excretion of 17 KGS, with a maximum within the 24 hours after the administration of metopirone was seen (fig 1) The maximal excretion varied considerably from individual to individual and was from 81 to 569 (average 180) per cent higher than the average excretion of 17-KGS in the 48 hours preceding the metopirone administration Within the second 24 hours after the test dose, the excretion of 17-KGS again approached normal values There was no significant sex difference as far as the maximal increase in 17 KGS was concerned

Individuals with non-endocrine diseases

In 2 hospitalized patients with gastritis *increases in the urinary 17-KGS similar* to those of healthy individuals were seen after metopirone administration (table I)

Hypophysectomized patients

Of 6 patients with metastasizing breast cancer who at least 2 months prior to the

TABLE I Urinary steroid response to metopirone Urinary excretion of 17 ketogenic steroids (17 KCS) mg/24 hrs

	Sex	Age	Day −2	Day −1	Metopirone	Day +1	Day +2	Remarks	
Normal									
K H	♀	18	3 8	3 9	14 0	26 1	9 5		
T S	♂	23	12 8	13 7	18 5	28 4	20 1		
K G	♀	23	9 7	6 8	14 2	15 5	10 4		
B A	♀	27	10 8	12 4	15 7	21 0	12 3		
K J	♀	29	9 6	10 7	17 8	23 7	17 4		
C I	♂	29	11 4	11 8	10 1	27 6	13 9		
S H	♀	35	12 7	7 3	26 1	31 9	15 3		
E D	♂	37	11 2	9 7	11 5	23 8	11 8		
J D	♂	37	14 8	14 3	30 2	42 0	18 8		
P H	♂	45	13 6	12 0	23 9	31 4	16 9		
Non-endocrine disease									
C T		38	—	9 3	19 8	39 6	—	Gastritis	
A J		47	—	9 0	14 6	23 6	—	Gastritis	
Hypophysectomy								Plasma compound day before metopirone intake	S µg/l day after metopirone intake
E N		48	—	4 7	4 9	3 4		11	17
E G		61		2 0	2 4	2 3		12	16
P H		50	—	2 8	3 0	2 3		24	19
A H		62	—	4 3	5 3	4 0		15	12
H B		43		1 4	2 0	3 7		6	33
F J		41		2 7	1 0	4 4		2	30
Chromophobe adenoma								ACTH test	
C J		40	2 1	1 1	2 0	1 3	1 6	Baseline 2 9 after ACTH 18 6	
F H		35	10 2	7 9	9 4	9 4	13 2	Baseline 10 2 after ACTH 49 9	
Adrenalectomy									
F S		38		14 7	3 9	4 4	—	During withdrawal of steroids	
K C		34	18 3	24 3	21 1	27 1	20 9	On cortone acetate	
Cushing's syndrome due to adrenal hyperplasia									
F H		20	15 2	14 4	27 0	45 5	33 3		
K N		31	20 8	22 2	25 9	49 9	22 [illegible]		
L J		45	16 2	18 4	23 1	29 3	20 6	After pituitary irradiation	
Diabetes mellitus									
C F		31		11 9	16 0	20 5		After acidosis	
P H		12	6 5	10 8	11 8	20 3	10 1	After acidosis	

TABLE II Treatment with steroids

	Sex	Age	Day −2	Day −1	Metopirone	Day + 1	Day + 2
Exogenous corticosteroids							
H W	♂	52	–	16.1	18.8	11.2	–
H I	♀	29	–	14.7	14.2	12.7	12.9
B M	♀	37	4.8	4.8	5.6	6.9	6.1
I M	♀	39	11.5	9.2	12.6	4.7	4.1
P Q I	♀	51	7.3	6.5	6.3	7.5	10.8
P Q II	♀	51	3.6	5.3	8.2	14.4	6.0
G O	♀	23	–	8.2	14.0	30.3	–
Exogenous ACTH							
C I	♀	64	–	43.7	36.9	46.5	41.3
I P	♀	68	–	6.3	7.5	13.9	8.6
I B	♀	39	9.4	15.0	19.2	45.5	14.6

metopirone test had undergone a trans sphenoidal hypophysectomy, and who for a short period at the time of the test were without substitution therapy with steroids, 4 showed no increase in the urinary excretion of 17-KGS or the amount of Compound S in the plasma after metopirone administration (fig 2) Two hypophysectomized patients (H B and F J) did, however show an increase both of the urinary excretion of 17-KGS and especially of Compound S in plasma after metopirone which indicated that the hypophysectomy was incomplete

Chromophobe pituitary adenoma

In 2 patients with chromophobe pituitary adenoma both of whom had been treated with subtotal resection metopirone administration did not give rise to an increase of the excretion of 17-KGS (fig 3) Neither of the patients showed signs of myxoedema or had had symptoms of insufficiency of the adrenal cortex, in spite of the fact that the

Diagnosis	Therapy
Purpura thrombocytopenica	Prednisone 90 mg/day for 29 days and 30 mg/day for 52 days
	Test during steroid treatment
Lupus erythematosus diss	Prednisone 30 mg/day for 12 months
	Test during steroid treatment
mb Addisonii obs pro	Cortisone 50 mg/day for 11 months
	Test 14 days after therapy had ended. ACTH test—baseline 7 2 after ACTH 22 4
mb Addisonii obs pro	Cortisone 50 mg/day for 12 months
	Test 14 days after therapy had ended ACTH test—baseline 2 9 after ACTH 18 6
Hypopituitarism obs pro	Prednisone 10 mg/day for 27 days
	Test 12 days after therapy had ended ACTH test—baseline 2 9 after ACTH 18 6
	Test 6 months after therapy had ended
Rheumatoid arthritis	Prednisone 10 mg/day for 25 days
	Test 10 days after therapy had ended
Asthma bronchiale	ACTH 60 I U /day for 9 days ACTH 30 I U /day for 5 days
	Test during ACTH treatment
Asthma bronchiale	ACTH 40 I U /day for 8 days
	Test 20 days after therapy had ended
Asthma bronchiale	ACTH 20 I U /day for 3 days
	Test 7 days of therapy had ended

spontaneous excretion of 17 KGS in one patient (Aa J) was subnormal A subsequent ACTH test gave rise to a normal increase in the excretion of 17 KGS which showed that the response of the adrenal glands was normal

Adrenalectomized patients

With the metopirone test carried out in 2 bilaterally adrenalectomized patients there was the expected lack of a rise in the excretion of 17 KGS (fig 3) One patient K G) was before and during the test given substitution therapy with 50 mg cortisone acetate orally whereas the metopirone test in the other patient (E A) took place during the tapering off of the cortisone dosage

Cushing s syndrome

Metopirone tests carried out in 2 patients with untreated Cushing s syndrome (E H and K N (fig 4)), in whom the diagnosis later was confirmed by operation showed normal percentage increases of urinary 17 KGS In the

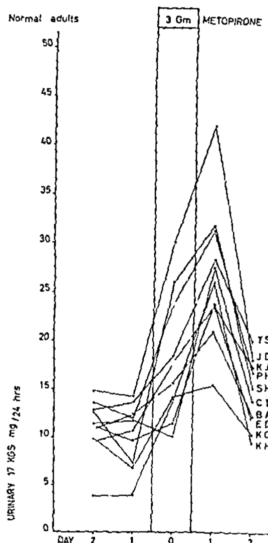


Fig 1 Normal individuals

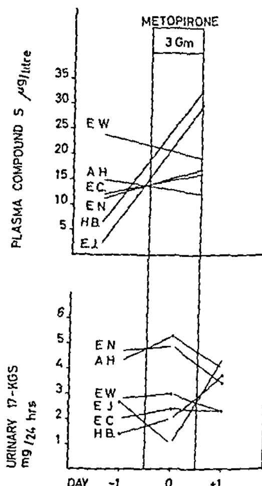


Fig 2 Hypophysectomized patients

third patient (I J), who 2 years previously had been treated with X-ray therapy to the pituitary gland, but who at the time of the test showed signs of recurrence, the metopirone test gave a subnormal increase (29 %) in the 17-KGS excretion

Diabetes mellitus after acidosis

In 2 juvenile diabetic patients the metopirone test was carried out immediately after several days of diabetic acidosis (blood sugar approx 400 mg/100 ml and plasma bicarbonate 18 mEq/l) In patient (G F) a subsequent increase in the excretion of 17 KGS was subnormal (72 %) while the metopirone test in the other patient (P H) was normal

Steroid-treated patients

Tables I—II and fig 5 show the result of metopirone test carried out in 6 patients during or some time after treatment with steroids In 2 patients (H W,

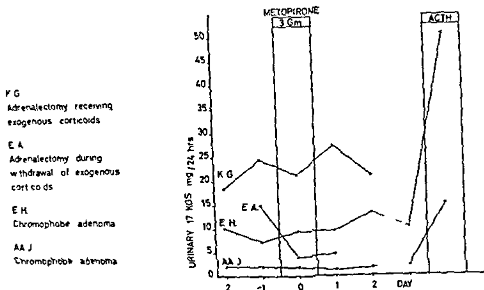


Fig 3 Adrenalectomized patients

H I) in whom the test was carried out during long term treatment with relatively high doses of prednisone the metopirone test was negative which indicated suppression of the pituitary adrenal function

In 2 patients (B M, F M) who approximately 12 months previously in other hospital departments were started on cortisone treatment for suspected Addison's disease the metopirone test carried out 14 days after withdrawal of steroid treatment showed in both a negative test while an ACTH test was normal The result thus showed an inhibited pituitary function with a normal response from the adrenal cortex and thereby excluded Addison's disease in these patients

In one patient (P Q) with suspected Simmond's cachexia the metopirone test was carried out 12 days after a 27

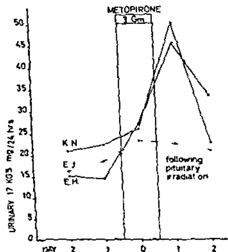


Fig 4 Cushing's syndrome

day period of prednisone medicamentation The test was negative thus showing an inhibition of the endogenous ACTH secretion A subsequent ACTH

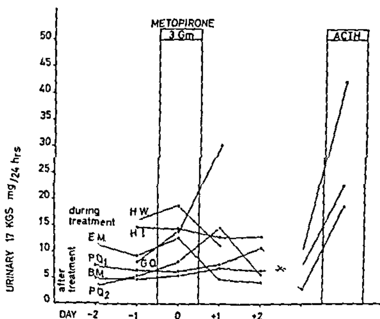


Fig 5 Steroid treated patients

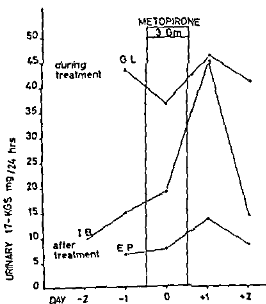


Fig 6 Corticotrophin treated patients

test was normal A repeated metopirone test carried out 6 months after withdrawal of prednisone was, however, normal, and this result thus excluded the diagnosis of Simmond's cachexia

In one patient (G O), who for 25 *days had received 10 mg prednisone* daily, the metopirone test, carried out 10 days after withdrawal of steroids, was normal

Corticotrophin treated patients

The result of the metopirone test carried out in 3 patients during or shortly after treatment with ACTH, is seen in table II and fig 6 In 1 patient (G L) where the test was carried out during ACTH treatment, the metopirone test did not give rise to an increase in the excretion of 17-KGS, which indicated an inhibition

of the endogenous ACTH secretion capacity In 2 patients (E P and I B) in whom the metopirone test was carried out 20 and 7 days respectively after a short period of ACTH treatment, the test was normal

Discussion

These results of the metopirone test, carried out in varied clinical conditions with confirmed or suspected disturbances in the pituitary adrenal function, illustrate the value of this test in assessment of the pituitary capacity for secreting ACTH Metopirone will under normal conditions, by inhibiting the cortisol synthesis induce an increased release of corticotrophin from the pituitary gland This compensatory" ACTH secretion gives rise to a corresponding release of corticosteroid precursors especially Compound S from the adrenal cortex, as followed in this investigation by determination of Compound S in plasma or of the urinary 17 ketogenic steroids The reason why the determination of 17 KGS excretion has been preferred is that the increase of the 17 KGS excretion after metopirone administration is almost twice as large as that of the 17 hydroxycorticosteroids (8)

The tests carried out in normal individuals show a marked increase in the 17 KGS-excretion on the day after metopirone administration and the increases are at least 80 % of the spontaneous values A considerable individual variation from person to person is noticed This finding is not surprising in that the metopirone test is an indirect test in which a variety of factors influence the result Among such factors are the conditions with regard to absorption of metopirone, the completeness of the inhibition of cortisol synthesis, the rate by which the plasma concentration of cortisol is reduced, the adrenocortical response to graded doses of ACTH, as well as the rate of metabolism and conditions with regard to excretion of Compound S and its metabolites Because of these varying conditions the metopirone test can only give a rough assessment of the pituitary function, and small deviations from the normal values can hardly be considered of definite pathological significance

Metopirone test carried out in hypophysectomized patients show in most cases the expected result i e clearly negative tests In 2 patients in whom no pituitary gonadotrophin could be demonstrated in the urine, considerable increases in the amount of Compound S in plasma were, however seen after administration of metopirone Such a reaction depends on the presence of cells capable of secreting ACTH Post mortem examination in one of these patients (H B) confirmed this by the finding of remaining pituitary tissue The metopirone test thus seems to be of use for detecting cases where hypophysectomy has been incomplete (6 7)

It is of interest that patients with chromophobe pituitary adenoma had negative metopirone tests even in cases with a normal basic excretion of 17 KGS These findings support the assumption that corticotrophin secretion is regulated by at least two different mechanisms One centre presumably localized in the hypothalamus mobilizes

corticotrophin under the indirect influence of stress or metopirone, whereby the level of cortisol is increased above normal, and another centre, possibly pituitary, has the primary task of maintaining a basic cortisol level in the plasma. It appears likely that the sensitivity of the first mechanism can be reduced even by limited changes in the pituitary gland. It is, on the other hand, known that 70—90 % of the hypophysis has to be damaged before the basic cortisol level of the blood starts to fall (18). It therefore seems likely that case E. M., who showed a reduced capacity for ACTH secretion and a normal basic 17-KGS secretion, had far less pituitary destruction than case A. J., who showed both a negative metopirone test and a low basic 17-KGS excretion.

As found by other investigators (9), patients with Cushing's syndrome due to hyperplasia of the adrenal cortex showed normal increases in 17-KGS excretion. This is contrary to the findings in Cushing's disease due to carcinoma or adenoma in the adrenal cortex, where no increase of the 17-KGS excretion is observed after metopirone (11). By a *single test with metopirone* the differential diagnosis between hyperplasia and tumour of the adrenal cortex can therefore be established.

As in previous investigations (1, 14—16), a loss of the capacity for secretion of ACTH from the pituitary gland was found in patients under treatment with corticosteroids or corticotrophin. Moreover, in several cases where the metopirone test was carried out shortly after discontinuation of the long-term treatment with steroids, an inhibited ACTH secretion was seen. A subsequent ACTH-test showed in all cases a normal increase in the secretion of 17-ketogenic steroids, which indicated a normal adrenal cortical function. In patients who are due to undergo an operation and who shortly before have received long term treatment with steroids, a *metopirone test can therefore be employed* for assessment of the indication for supportive corticosteroid therapy. A normal metopirone test in such patients shows normally reacting pituitary and adrenals and supportive therapy is unnecessary. A negative metopirone test does not necessarily mean that an operation gives rise to development of insufficiency of the adrenal cortex, but a "steroid umbrella" will in such cases seem reasonable and advisable (5, 9).

A few patients have noticed nausea or headache on the day of or following the administration of metopirone. No signs of shock or insufficiency of the adrenal cortex appeared in any of the cases and the risk of shock must be considered low in relation to the large number of patients investigated with metopirone. In the literature only one case seems to be reported (14). Even the administration of metopirone for months is well tolerated (19). If the metopirone test is carried out in hypophysectomized patients under withdrawal of steroids, the patient must be closely observed with regard to shock and cortisol hemisuccinate must be at hand.

Summary

Standardized peroral metopirone tests have been carried out in 36 individuals of whom 24 had confirmed or suspected

disturbances of the pituitary adreno cortical function

In normal individuals an increase in the urinary excretion of 17 KGS of at least 80 % of the spontaneous excretion was seen within 24 hours after the administration of metopirone

Hypophysectomized patients all showed a low spontaneous excretion of 17 KGS In 2 patients without demonstrable pituitary gonadotrophin in the urine metopirone gave rise to a slight increase in the urinary excretion of 17 KGS and a marked increase in the amount of 11 desoxycortisol in plasma This finding indicates that the test can detect cases of incomplete hypophysectomy

In cases of chromophobe pituitary adenoma and in adrenalectomized patients the metopirone test was negative

Cases of Cushing s syndrome due to hyperplasia of the adrenal cortex showed a normal metopirone test with the exception of one case who previously had been treated with irradiation of the hypophysis Corticosteroid or corticotrophin treated patients or patients in whom long term steroid treatment had been discontinued shortly before the test showed negative metopirone tests It is concluded that the metopirone test is suitable for an assessment of the hypothalamic pituitary mechanism which controls the release of ACTH and thereby for the assessment of hypofunction of the pituitary gland

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corticotrophin under the indirect influence of stress or metopirone, whereby the level of cortisol is increased above normal, and another centre, possibly pituitary, has the primary task of maintaining a basic cortisol level in the plasma It appears likely that the sensitivity of the first mechanism can be reduced even by limited changes in the pituitary gland It is, on the other hand, known that 70—90 % of the hypophysis has to be damaged before the basic cortisol level of the blood starts to fall (18) It therefore seems likely that case E M, who showed a reduced capacity for ACTH secretion and a normal basic 17-KGS secretion, had far less pituitary destruction than case A J, who showed both a negative metopirone test and a low basic 17-KGS excretion

As found by other investigators (9), patients with Cushing's syndrome due to hyperplasia of the adrenal cortex showed normal increases in 17-KGS excretion This is contrary to the findings in Cushing's disease due to carcinoma or adenoma in the adrenal cortex where no increase of the 17-KGS-excretion is observed after metopirone (11) By a single test with metopirone, the differential diagnosis between hyperplasia and tumour of the adrenal cortex can therefore be established

As in previous investigations (1 14—16), a loss of the capacity for secretion of ACTH from the pituitary gland was found in patients under treatment with corticosteroids or corticotrophin Moreover, in several cases where the metopirone test was carried out shortly after discontinuation of the long term treatment with steroids, an inhibited ACTH secretion was seen A subsequent ACTH test showed in all cases a normal increase in the secretion of 17-ketogenic steroids, which indicated a normal adrenal cortical function In patients who are due to undergo an operation and who shortly before have received long-term treatment with steroids, a metopirone test can therefore be employed for assessment of the indication for supportive corticosteroid therapy A normal metopirone test in such patients shows normally reacting pituitary and adrenals, and supportive therapy is unnecessary A negative metopirone test does not necessarily mean that an operation gives rise to development of insufficiency of the adrenal cortex, but a "steroid umbrella" will in such cases seem reasonable and advisable (5, 9)

A few patients have noticed nausea or headache on the day of or following the administration of metopirone No signs of shock or insufficiency of the adrenal cortex appeared in any of the cases and the risk of shock must be considered low in relation to the large number of patients investigated with metopirone In the literature only one case seems to be reported (14) Even the administration of metopirone for months is well tolerated (19) If the metopirone test is carried out in hypophysectomized patients under withdrawal of steroids the patient must be closely observed with regard to shock and cortisol-hemisuccinate must be at hand

Summary

Standardized peroral metopirone tests have been carried out in 36 individuals of whom 24 had confirmed or suspected

disturbances of the pituitary adrenocortical function

In normal individuals an increase in the urinary excretion of 17 KGS of at least 80 % of the spontaneous excretion was seen within 24 hours after the administration of metopirone

Hypophysectomized patients all showed a low spontaneous excretion of 17 KGS In 2 patients without demonstrable pituitary gonadotrophin in the urine, metopirone gave rise to a slight increase in the urinary excretion of 17 KGS and a marked increase in the amount of 11 desoxycortisol in plasma This finding indicates that the test can detect cases of incomplete hypophysectomy

In cases of chromophobe pituitary adenoma and in adrenalectomized patients the metopirone test was negative

Cases of Cushing s syndrome due to hyperplasia of the adrenal cortex showed a normal metopirone test, with the exception of one case who previously had been treated with irradiation of the hypophysis Corticosteroid or corticotrophin treated patients or patients in whom long term steroid treatment had been discontinued shortly before the test showed negative metopirone tests It is concluded that the metopirone test is suitable for an assessment of the hypothalamic pituitary mechanism which controls the release of ACTH and thereby for the assessment of hypofunction of the pituitary gland

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Digestive Tract in Collagen Diseases

By

M Siurala, H Julkunen, S Toivonen R Pelkonen, E Saxen and E Pitkanen

Various disturbances of the digestive tract have been described in collagen diseases (2 6 7 8 12 20 21 30 31) Scleroderma in particular seems to be associated with alterations of the upper gastrointestinal tract (1 4, 5, 9, 10, 11, 14 15 16 22 24 26 27) Recently Toivonen et al (29) have described two patients suffering from collagen disease other than scleroderma, which was associated with malabsorption and with changes in the mucosa of the small intestine characteristic of adult coeliac disease

This report deals with results of gastroenterological examinations performed on 71 consecutive patients with collagen disease

Material and methods

This study was carried out at the I and II Medical Departments of the University Central Hospital Helsinki and lasted from autumn 1962 to autumn 1964 All patients who had symptoms and signs of joint muscular cardiac vascular and serosal involvement and had an increase in the blood immunoglobulins were considered to suffer from collagen disease and were included in the series However some patients who fulfilled these criteria either declined gastroenterological examinations or these examinations could not be performed because of the poor condition of the patient There remained 71 patients for gastroenterological examinations

The mean age of the patients was 47 5 years (range 18—68 years) There were 47 females and 24 males

The distribution of the patients as to the kind of collagen disease is indicated in table I

All the 14 patients with undetermined collagen disease fulfilled the criteria presented One of them had arthritis with Sjogrens syndrome and one temporal arteritis

Corticosteroid treatment was given to 27 patients before and during the time of the gastroenterological examinations

The patients suffered from the following concomitant diseases hyperthyroidism (2) hypothyroidism (1 diabetes mellitus (2) hypertension (2) congestive heart failure (1) and bronchiectasis (1)

Two control materials were used one for anatomical changes in the small intestine and the gastric mucosa and one for exocrine

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TABLE III Histological state of the gastric mucosa

	Rheum arthritis	SLE	Other defined collagen dis	Undefined collagen dis	Controls[4]
Normal	2	5	4	5	12
Superficial gastritis[1]	5	4	—	2	4
Slight and moderate atrophic gastritis[2]	3	6	1	2	2
Severe atrophic gastritis[3]	5	2	1	—	2
Total	15	17	6	9	20

[1] Significant inflammatory cell infiltration below the surface epithelium
[2] Slight or moderate loss of normal body glands
[3] Severe or total loss of normal body glands
[4] Patients with adult coeliac disease

TABLE IV Anatomical state of the small intestine

	Rheum arthritis	SLE	Other defined collagen dis	Undefined collagen dis
Total or subtotal villous atrophy	—	2	—	4
Minor epithelial changes	—	1	1	—
Increase of inflammatory cells without epithel alterations	4	4	—	1
Normal	14	12	3	5
Total	18	19	4	10

patients suffered from abdominal pain Of these 16 had attacks of pain 14 epigastric postprandial pain and 2 postprandial pain elsewhere Six patients complained of diarrhoea 5 of them had intestinal malabsorption

Of the patients suffering from abdominal pain 4 had gallbladder disease 5 chronic pancreatitis 2 active gastric ulcer 2 gastric ulcer in the past 1 hiatal hernia 1 cancer of the colon and 3 intestinal malabsorption In the remaining 14 patients no obvious cause for the pain was found Two patients underwent operation for acute abdomen However no explanation for the pain was found at operation

The occurrence of abdominal pain seemed to bear no relationship to the intake of analgesic and anti inflammatory drugs e g abdominal pain was present in 38 % of patients treated with corticosteroids and in 39 % of those not taking these drugs

TABLE I Distribution of the patients as to the kind of collagen disease

	No
Rheumatoid arthritis (positive Waaler Rose test and typical clinical picture)	20
Rheumatoid spondylitis (typical vertebral involvement)	3
Systemic LE (LE cell phenomenon and DNA antibodies present with or without typical clinical picture)	28
Dermatomyositis (characteristic histology)	3
Scleroderma (characteristic histology)	2
Polyarteritis nodosa (characteristic histology)	1
Undetermined collagen disease	14
Total	71

TABLE II The occurrence of abdominal pain

	Rheum arthritis	SLE	Other defined collagen dis	Undefined collagen dis
Epigastric pain	5	5	1	3
Pain elsewhere	1	1	–	–
Pain attacks	4	2	4	6
No pain	10	20	4	5
Total	20	28	9	14

function of the pancreas The selection of the control series will be given in the following chapters

In addition to the physical examination a blood smear, routine analysis of the urine electrophoretic analysis ESR LE cell phenomenon DNA antibody titer Waaler Rose test, latex test and Wassermann test were performed on all patients

The following gastroenterological examinations were performed

Small intestinal biopsy using the multi purpose suction biopsy tube in 51 patients Specimens were taken from the distal duodenum somewhat proximal to the ligament of Treitz The specimens were flattened out on a piece of paper, examined with a lupe, fixed for 24 hours with formalin, and then cut perpendicular to the surface and sent to the pathological laboratory The specimens were stained with Hematoxylin eosin Hematoxylin v Gieson and Periodic Acid Schiff stains

Gastric biopsy using Siehf s suction biopsy tube One hundred and twenty five specimens of the gastric body mucosa were obtained from 47 patients The specimens were fixed and stained in the same way as the small intestinal specimens

Quantitative chemical fecal fat determination by the method of van de Kramer on 26 patients

Peroral glucose tolerance test on 28 patients (glucose 1 g/kg body weight)

Vitamin A tolerance test on 50 patients (blood examinations before and 5 hours after a 10 000 U /kg vitamin A)

d Xylose tolerance test on 64 patients (d xylose content of 5 hours urine after a peroral dose of 25 g, pathological values were taken into consideration only if the amount of urine exceeded 500 ml)

Ordinary Schilling test in 38 patients (24 hours urinary radioactivity after peroral dose of 0 6—0 7 γ of Co^{60}-vitamin B_{12})

Serum calcium and serum iron determinations on 50 patients

Liver function tests serum glutamic oxalic acid transaminase bromsulphthalein retention and serum alkaline phosphatase on 64 patients Needle biopsy of the liver was performed on 11 patients

Exocrine function of the pancreas using duodenal intubation with secretin stimulation (Dreiling and Janowitz) on 31 patients

X ray examination of the stomach and small intestine on 53 patients

Results

1 Abdominal complaints

The occurrence of abdominal pain is presented in table II Thirty two of 71



Fig 4 Patient A S with undefined collagen disease and malabsorption Male 52 years Decreased pancreatic but normal liver function Gastric mucosa normal Histologically there is a total loss of villi and severe inflammatory changes H E x 90



Fig 6 Patient F K with adult coeliac disease without systemic signs Histologically there is subtotal loss of villi with severe inflammatory changes H E x 90



Fig Patient L H with systemic LE and malabsorption Male ? years Liver function normal Iron deficiency anemia Histologically there is total loss of villi and severe inflammatory changes H E x 90

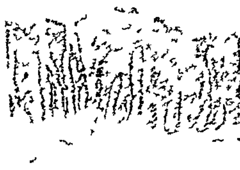


Fig 7 Patient I L with adult coeliac disease without systemic signs Histologically the same changes are seen as in figs 4 and H E x 90

c tr ls These characteristics of patients with collagen disease might have contributed to the high incidence of gastritis as gastritis is said to be more common in females and to increase with advancing age On the other hand in the present series of collagen disease there was no increase of gastritis with age the mean age of patients with a normal mucosa being 47 years and that of gastritis 43 years Moreover the incidence of gastritis in the collagen group was somewhat lower in females than in males Hence it would appear that the incidence of gastritis in patients with collagen disease was in the main independent of the sex and age distribution

The possible effect of drug therapy upon the occurrence of gastritis was also evaluated However the use of analgesics and corticosteroids seemed to bear *no* clear relationship to the occurrence of gastritis Thus the incidence of gastritis



Fig 1 Patient V I with rheumatoid arthritis Male 57 years Intestinal absorption normal Liver function pathological Chronic gastritis and pancreatitis Histologically the intestinal mucosa looks normal H E x 90



Fig 3 Patient A S with systemic LE Female 16 years Intestinal absorption and liver function normal Gastric mucosa normal Serum calcium and iron levels low Histologically there is some shortening of the villi and an increase in the number of inflammatory cells H E x 90



Fig 2 Patient L D with polyarteritis nodosa Female 59 years Intestinal absorption and pancreatic and liver function normal Gastritis Histologically the number of inflammatory cells is increased but the epithelium appears nearly normal H E x 90

2 *State of the stomach*

X-ray examination of the stomach revealed hiatal hernia in one and gastric ulcer in 2 patients None of the ulcer patients had received corticosteroids

Gastric biopsy was performed on 47 patients The results are shown in table III The results were compared with the biopsy findings in 22 consecutive patients suffering from adult coeliac disease without systemic manifestations Gastritis was found in 31 (66 %) of the 47 patients with collagen disease, and in 8 (36 %) of 22 controls However the two groups are not quite comparable as to age and sex distribution The mean age of patients with collagen disease was 47 years and that of controls 36 years The male — female ratio was 0 5 in the collagen group" and 0 7 in the

figs 4 and 5)

Vit A toler test	d Xylose toler test (g/5 h)	Schilling test (o)	Maximal bicarb output (mEq/l)	Liver function	Gastric biopsy	Intestinal biopsy	Interpretation
Pathol	1 4	15 7	37	Normal	Normal	Total loss of villi	Undefined collag dis with malabs
Pathol	3 9	14 0	—	Pathol	Superf gastritis	Total loss of villi	Undefined collag dis with malabs
Pathol	2 6	*0	—	Normal	—	Total loss of villi	Undefined collag dis. with malabs
Pathol	1 5	5 5	-	—	—	Total loss of villi	Polyarteritis and polymyositis with intest. malabs
Pathol	2 1	*6 7	—	Normal	—	Total loss of villi	SLE with intest malabs.
Pathol	2 3	7 6	31	Normal	Superf gastritis	Subtotal loss of villi	SLE with intest malabs
Pathol	1 9	8 0	—	Pathol	—	—	SLE with intest malabs

*No increase with intrinsic factor

and 5) and 2 minor changes (fig 2) There was a great increase of inflammatory cells in these 8 patients with epithelial alterations An additional 9 patients showed increased amounts of inflammatory cells (fig 3) without distinct epithelial changes The most common cell types were plasma cells and lymphocytes Plenty of eosinophils were seen in specimens of 18 patients Corticosteroid treatment given to 15 of the 51 patients seemed to have some effect upon the composition of the lamina propria Thus distinct eosinophilia was seen in 2 of 15 patients treated with corticosteroids but in 16 of 36 patients who had not received this treatment

The changes in lamina propria muscularis mucosae and submucosa of patients with collagen disease were compared with those seen in 22 consecutive patients with adult coeliac disease (figs 6 and 7) However no essential difference was found between the groups under comparison Thus no changes characteristic of collagen disease could be found in specimens of patients with collagen disease

On the other hand submucosal edema was found in one third of the

TABLE V Gastroenterological findings in 7 patients with collagen disease and malabsorption (cf

Patient	Age Sex	Case history	Waaler Rose Latex LE cells DNA antib	ESR (mm/1 h)	Hb Er	Serum iron	Serum calcium (mg/100 ml)	Faecal fat (g/day)
A S	52 ♂	Diarrhoea and joint pain Pericarditis	Normal	50	9 6 3 3	11	8 5	12
O R	52 ♀	Diarrhoea and joint swelling	Normal	70	11 6 3 8	103	9 1	–
L A [1]	54 ♀	Diarrhoea and joint swelling	Latex pos	100	8 0 2 4	29	9 2	12
S W [1]	51 ♂	Fever, muscle pain diarrhoea Myositis poly arteritis	Normal	105	10 0 3 2	4	8 9	70 % of dry subst
L H	54 ♂	Weakness, oedema	LE cells	105	9 8 3 6	23	9 0	10
M G	36 ♀	Fever joint pain, abd pain	LE cells DNA antib	127	8 4 2 9	31	7 7	0 8
H F	35 ♂	Joint swelling Abd pain	Waaler Rose pos LE cells DNA antib	102	10 2 3 9	48	7 4	7 2

[1] Described in detail elsewhere (29)

in patients treated with corticosteroids was almost the same as in other patients

Closer examination of the biopsy specimens revealed intestinal metaplasia and pseudopyloric metaplasia in 8 and pseudopyloric metaplasia alone in 12 patients The inflammatory" changes were in general pronounced Large numbers of granulocytes were seen in more than a half of the patients Numerous eosinophils were found in approximately 60 % of patients not treated with corticosteroids The predominating cell types were, however, plasma cells and lymphocytes The latter were found in almost all specimens with gastritis in large aggregates with a germinal center

No histological changes suggestive of collagen disease could be found in any of the gastric biopsy specimens In specimens of two patients there was a scar like increase of connective tissue between the tubules

3 *Anatomical state of the small intestine*

The results of histological examinations of intestinal specimens are shown in table IV Of 51 patients 6 showed total or subtotal atrophy of the villi (figs 4

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3 *Anatomical state of the small intestine*

The results of histological examinations of intestinal specimens are shown in table IV *Of 51 patients 6 showed total* or subtotal atrophy of the villi (figs 4

TABLE VI Maximal bicarbonate output after secretin stimulation

Bicarbonate output (mEq/l)	Rheum arthritis	SLE	Other collag	Controls
< 50	1	2	2	0
50—70	2	1	—	0
> 70	8	6	9	20
Total	11	9	11	20

the gastroenterological examinations Hence, it is possible that treatment with corticosteroids might to some degree have masked the signs of intestinal malabsorption

No correlation could be shown to exist between the severity of collagen disease and the results of absorption tests

5 *Exocrine function of the pancreas*

Duodenal intubation with secretin stimulation was performed on 31 patients The results of absorption tests are shown in table VI

Twenty consecutive patients in the outpatient department of the II Department of Medicine who had no abdominal complaints served as controls The lowest bicarbonate concentration found in controls was 70 mEq/l Of the patients with collagen disease 8 had bicarbonate values below 70 mEq/l An amylase activity below 500 units was found in 7 patients and 2 controls and a decreased volume in 7 patients and 4 controls

Two of the 8 patients with decreased bicarbonate output had periodic abdominal pain with increased urinary amylase activity An additional 3 patients, in whom duodenal intubation was not performed had clinical signs of relapsing pancreatitis Thus chronic pancreatitis with clinical manifestations was found in a total of 5 patients

The occurrence of pancreatic lesions seemed to bear no relationship to treatment with corticosteroids Neither was there any correlation between the severity of collagen disease and the exocrine function of the pancreas

6 *State of the liver*

The function of the liver was studied in 64 patients Liver biopsy was performed in 11 patients

Increased bromsulphthalein retention (above 5 % in 45 min) was found in 22 increased activity of glutamic oxalic acid transaminase (above 40 Sigma Fraenkel units) in 10 and increased activity of alkaline phosphatase (above 10 King Armstrong units) in 16 of the 64 patients examined All these tests were positive in 10 and negative in 31 of the 64 patients

Liver biopsy revealed normal structure (3) acute hepatitis (3) fatty liver (3) and chronic hepatitis (2)

patients with collagen disease, and mainly in patients who were treated with corticosteroids However, the possibility that edema might be an artefact caused by suction cannot be excluded

Hematoxylin bodies were not found in patients with systemic LE PAS stain revealed no signs of Whipple's disease

4 *Functional state of the small intestine*

X ray examination of the small intestine was performed on 53 patients and revealed deficiency pattern" in 5

Signs of intestinal malabsorption were found in 7 patients Small intestinal biopsy was performed on 6 of these and revealed total or subtotal loss of the villi Three of the patients suffered from systemic LE and the remaining from a collagen disease which could not be closer identified The results of various gastroenterological examinations performed on these 7 patients are listed in table V

Three patients with intestinal malabsorption were treated with gluten free diet and 4 with corticosteroids Of the 3 patients treated with gluten free diet 2 responded with symptomatic and objective improvement One of the 4 patients treated with corticosteroids died shortly after the beginning of treatment one did not respond to treatment and 2 declined further examinations

In the 4 patients treated with corticosteroids the signs of collagen disease clearly dominated the clinical features and seemed to precede the signs of malabsorption On the other hand the signs of malabsorption were predominant in the 3 patients treated with a gluten free diet

In addition to the 7 patients with intestinal malabsorption, 13 patients showed pathological values in one of the following tests d xylose tolerance test vitamin A tolerance test, glucose tolerance test and Schilling test The chemical fecal fat determination revealed pathological values only in patients with distinct intestinal malabsorption

Schilling test was below 5 % of the given dose in 4 patients Additional 6 patients had low normal values (5—10 %) One patient had megaloblastic anemia which responded to treatment with vitamin B_{12} Addition of intrinsic factor did not increase the urinary excretion of radioactivity However, one patient had achlorhydria and complete loss of normal gastric body glands suggesting that intrinsic factor deficiency might also have been involved in this case

Serum iron was below 50γ 100 ml in 29 of 50 patients Of the 29 20 had in addition total iron binding capacity values above 350γ/100 ml Iron deficiency anemia was present in 14 patients It could not be decided whether iron deficiency was due in the main to malabsorption of iron or to factors in some way inherent to collagen disease

Serum calcium was below 8 mg 100 ml in 8 and between 8 and 9 mg 100 ml in 15 of 50 patients Clinical signs of calcium deficiency were present in 2 patients

Serum proteins were below 6 % in 8 patients 5 of whom had signs of intestinal malabsorption

Pathological absorption tests were noted in the main in patients who had not received corticosteroids before or during

thorough examination of the biopsy specimens, no signs could be found definitely attributable to collagen disease

According to the third possibility there would be a simultaneous occurrence of adult coeliac disease and collagen disease, possibly on a common hereditary and autoimmune basis This seems to be supported by the fact that morphological changes in the small intestine of our patients were identical with those in the patients with adult coeliac disease Further, the response to gluten free diet in two patients with collagen disease suggests the presence of true coeliac disease

It could be thought, according to Burnet (18) that forbidden clones have led to autoimmunization against mucosal elements on one hand, and against mesenchymal tissue elsewhere on the other hand This is supported by some facts Hereditary factors are of importance in the development of collagen diseases as well as of coeliac disease Immunological phenomena occur in both diseases Antibodies against gastrointestinal mucosa have been demonstrated by some authors (17 19) Auto antibodies against gluten have been found in coeliac disease (3 13 28) In all our patients with collagen disease and malabsorption as well as in the patients with adult coeliac disease there were in the mucosa large numbers of lymphocytes and plasma cells which are assumed to be the site of antibody production Increased numbers of these cells were also found in many patients with collagen disease without epithelial alterations Possibly the occurrence of these antibody producing cells is the first sign of an immunological response of the gastrointestinal mucosa

Summary

Symptoms and signs of the upper digestive organs were studied in 71 patients with collagen disease (rheumatoid arthritis 20, rheumatoid spondylitis 3, systemic LE 28, dermatomyositis 3, scleroderma 2 polyarteritis nodosa 1, and undetermined collagen disease 14 patients) Two control series were used one for the study of histological changes in the gastrointestinal mucosa (22 consecutive patients with adult coeliac disease), and one for the study of the exocrine function of the pancreas (20 consecutive patients without abdominal complaint)

Thirty two patients suffered from upper abdominal pain 16 had pain attacks and 16 postprandial pain In 14 of these no obvious cause was found for the pain Two were operated on for acute abdomen but nothing pathological was found

X ray examination of the stomach and small intestine revealed hiatal hernia in 1, gastric ulcer in 2 and deficiency pattern in 5 of 53 patients

Gastric biopsy performed on 47 patients revealed gastritis in 31 superficial gastritis in 11, and atrophic gastritis in 20 patients Gastritis was present in 8 of 22 controls

Small intestinal biopsy performed on 51 patients revealed total or subtotal loss of villi in 6 minor epithelial changes in 2 and inflammatory' changes alone in 9 patients The commonest cell types here as well as in the gastric mucosa were plasma cells and lymphocytes

Discussion

The study showed that approximately half of the patients with collagen disease had some kind of abdominal complaint A great proportion of the patients showed epithelial alterations or only "inflammatory" reaction of the gastric and/or duodenal mucosa Many patients had decreased pancreatic function and functional or pathological changes in the liver Only 10 of the 71 patients examined revealed no changes in the upper digestive organs

However, many of the gastroenterological changes might have been purely coincidental or due to factors not directly related to collagen disease, such as treatment with antirheumatic drugs On the other hand, we could find no evidence that changes in the digestive organs were due to treatment with corticosteroids, the most commonly used drugs In contrast, it seemed possible that treatment with corticosteroids might have masked some functional and anatomical alterations of the small intestine Although the effect of treatment and possibility of pure coincidence cannot be excluded, the high incidence of gastroenterological symptoms and signs appears convincing

The occurrence of morphological alterations in the small intestine with concomitant malabsorption in collagen disease is of particular interest Assuming that some causal relationship exists between collagen disease and malabsorption, there are three possibilities to be considered for this coexistence Firstly, intestinal malabsorption is the primary disease secondly, collagen disease is the primary disease, and thirdly, collagen disease and malabsorption are parallel phenomena

As to the first possibility, it must be mentioned that in 4 of 7 patients with malabsorption the systemic signs dominated the clinical features, and that the intestinal signs seemed to appear later However, in three patients (A S, O R and L H, table V) most symptoms seemed to be due to malabsorption, which in two patients (A S and O R) responded adequately to treatment with gluten-free diet Hence the first possibility that malabsorption might be the primary disease should be considered in these two patients However, the systemic signs persisted in both patients in spite of a good response of the intestinal symptoms to gluten-free diet Moreover, it should be noted that Scudamore et al (25) could find no signs of systemic involvement in 272 patients with adult coeliac disease

The second possibility, that collagen disease might be the primary disease, appears more feasible Most of the intestinal mucosa consists of reticulo-endothelial and mesenchymal elements Moreover, the muscularis mucosa and submucosa are almost completely of mesenchymal origin Hence, it is possible that intestinal alterations represent only a part of the systemic mesenchymal reaction of the body In fact in 4 of 7 patients with intestinal involvement the signs of collagen disease were predominant and had preceded the symptoms of malabsorption The common involvement of other digestive organs might also support this view Direct evidence however, is lacking In spite of a

thorough examination of the biopsy specimens no signs could be found definitely attributable to collagen disease

According to the third possibility there would be a simultaneous occurrence of adult coeliac disease and collagen disease, possibly on a common hereditary and autoimmune basis This seems to be supported by the fact that morphological changes in the small intestine of our patients were identical with those in the patients with adult coeliac disease Further the response to gluten free diet in two patients with collagen disease suggests the presence of true coeliac disease

It could be thought according to Burnet (18) that forbidden clones have led to autoimmunization against mucosal elements on one hand and against mesenchymal tissue elsewhere, on the other hand This is supported by some facts Hereditary factors are of importance in the development of collagen diseases as well as of coeliac disease Immunological phenomena occur in both diseases Antibodies against gastrointestinal mucosa have been demonstrated by some authors (17, 19) Auto-antibodies against gluten have been found in coeliac disease (3 13 28) In all our patients with collagen disease and malabsorption as well as in the patients with adult coeliac disease there were in the mucosa large numbers of lymphocytes and plasma cells which are assumed to be the site of antibody production Increased numbers of these cells were also found in many patients with collagen disease without epithelial alterations Possibly the occurrence of these antibody producing cells is the first sign of an immunological response of the gastrointestinal mucosa

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Small intestinal biopsy performed on 51 patients revealed total or subtotal loss of villi in 6 minor epithelial changes in 2 and inflammatory changes alone in 9 patients The commonest cell types here as well as in the gastric mucosa were plasma cells and lymphocytes

Plenty of eosinophils were seen in specimens of 18 patients Of these only 2 had received corticosteroids, which seemed to have some effect upon the composition of the lamina propria of the small intestine In patients with collagen disease the lamina propria, muscularis mucosa and submucosa of both the stomach *and duodenum* showed essentially the same changes as in patients with adult coeliac disease

Intestinal malabsorption was found in 7 patients 3 of whom had systemic LE and 4 an undefined collagen disease Three of them were treated with a gluten free diet and 4 with corticosteroids Two patients responded adequately to the gluten free diet In addition to the 7 patients with intestinal malabsorption, 13 patients showed pathological values in one of the absorption tests performed

The ordinary Schilling test was below 5 % in 4 and between 5 % and 10 % in 6 of 38 patients Addition of intrinsic factor had no effect One patient had megaloblastic anemia

Serum iron values were below 50 γ/100 ml in 29, and TIBC above 350 γ/100 ml in 20 of 50 patients Iron deficiency anemia was present in 14 patients

Serum calcium was below 8 mg/100 ml in 8, and between 8 and 9 mg/100 ml in 15 of 50 patients

Duodenal intubation with secretin stimulation was performed on 31 patients and revealed a decreased bicarbonate output in 8 Five patients had clinical and laboratory signs of chronic pancreatitis

Treatment with corticosteroids given to 27 patients seemed to bear no clear relationship to the occurrence of abdominal pain, to changes in the gastric mucosa, and to changes in exocrine function of the pancreas

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From the Department of Clinical Physiology, Karolinska Institutet at Serafimerlasarettet
Stockholm Sweden

Exercise Electrocardiograms Recorded Twice with an 8-Year Interval in a Group of 204 Women and Men 48—63 Years Old

By

IRMA ÅSTRAND

In the Stockholm's City Health Survey of 1954 (8) a group of 40—55 year old subjects performed an electrocardiographic exercise test These subjects were re examined in 1962 The purpose of this study was to analyse these repeated ECG recordings Special attention has been given to ECG changes which may be used in the early diagnosis of coronary heart disease

Material

In the Stockholm's City Health Survey of 1954 randomly selected subjects of various ages participated Due to a large number of examination refusals the sample was not representative of the population of Stockholm but nevertheless represents a relatively good cross-section of this population When certain sub-samples were re-examined in 1962 the emphasis was placed upon individual comparisons 225 subjects participated in the section of the study reported in the present paper

Six subjects had symptoms of such severity at the time of examination that the exercise test was not carried out Of these 6 one had had one myocardial infarction after the first examination in 1954 One had atrial fibrillation with high ventricular rate The remaining 4 had no significant ECG changes but had diseases that made an exercise test impossible Four subjects were on daily digitalis therapy and thus excluded (15) Data from 11 subjects in 1954 could not be included in the analysis for technical reasons Thus the number of subjects with complete ECG data was reduced by 21 to a final total of 204 This final group consisted of 117 women and 87 men between 48—63 years of age in 1962 The mean age for both women and men was 53 5 years ($1 \times \sigma = \pm 3\ 0$)

Methods

After 15 minutes of rest in the supine position blood pressure was measured on the left arm with a cuff connected to a mercury manometer If the systolic and/or diastolic pressure was higher than 165 or 95 mm Hg respectively the readings were repeated at least twice and the lowest value used for computation

The subjects subsequently worked on a bicycle ergometer (Monark) with a pedalling frequency of 50/min The work

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The subjects subsequently worked on a bicycle ergometer (Monark) with a pedalling frequency of 50 min The work

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load was increased by steps with an exercise time of about 6 min at each level Most of the women began with a load of 300 kpm/min (oxygen uptake about 0,9 l/min), and the men at 600 kpm/min (oxygen uptake about 1,5 l/min) At the completion of each 6 minute period, the load was increased by 150 kpm/min for the women, and by 300 kpm/min for the men, until a heart rate of about 150/min had been reached, provided that no symptoms or ECG changes caused an earlier termination of the test

ECGs were recorded with a direct ink jet-writing 4 channel electrocardiograph (Mingograph 42 B, Elema Schonander AB, Stockholm) The following leads were used before exercise (lying) I, II, III, CR_1 CR_2, CR_4, CR_5, CR_7, aVR, aVL, aVF, during and immediately after exercise (sitting) CH_2, CH_4, CH_5, CH_7 (H = forehead), one, three and ten min after exercise (lying) I, II, III, CR_1, CR CR_4, CR_5 and CR_7

The ECG was recorded during exercise at the 2nd, 4th, 5th and 6th min at each load Heart rate was determined from the 4th, 5th and 6th min recordings, and the mean value was computed, except in the presence of a continuous heart rate increase In such cases and if there was an early discontinuation of exercise the last and the highest values were given

The subjects were requested to note carefully any symptoms occurring during the exercise test

ECG interpretation

Coding of the ECGs

All ECGs from 1954 and 1962 were classified according to the Minnesota code for the resting ECG (5) However, certain additions and modifications were made in regard to the S T segment and arrhythmias according to Blomqvist and Åstrand (6) S T J and S T segment amplitudes were measured from the level of the PR interval at the beginning of the QRS complex in leads I and II aVF CR_4, CR_5 and CR_7 The most extreme change was recorded The modified code is presented below Only items used in this material have been listed The reader is referred to Blackburn et al (5) for details The same code was used for the resting ECG as for the ECG during and after exercise Thus changes from rest to exercise were not coded as such (5) but all ECGs were coded independently, with use of the code originally designed for the resting ECG CR- and CH-leads agree very closely and are interchangeable (10) Whether the use of these leads instead of modified V-leads has resulted in any difference in classification is questionable

Code for the changes in the ECGs

I 0 No herein reportable electrocardiographic items

Q and QS patterns

I 2 Class II (5)

QRS axis deviation

II 1 Left QRS axis = —30° or greater (leads I, II and III)

High amplitude R waves

III 1 Left R + S>35 mm (CR_4 or CR_7) or R>20 mm (I II, III and aVF) or R>12 mm (aVL)

S T junction and segment (measured from preceding P R interval at onset of QRS leads I or II aVF, CR_{4-7}) (Modification of the original code)

IV 1 S T J depression 1 mm or more and S T segm horizontal or downward sloping

2 S T J depression 0 5—0 9 mm and S T segm horizontal or downward sloping

3 No S T J depression as much as 0 5 mm but S T segment sloping down reaching 0 5 mm or more below P R baseline

4 No S T J depression as much as 0 5 mm but S T segment horizontal or downward sloping but not reaching 0 5 mm below P R baseline

5 S T J depression 1 mm or more with normal configuration of S T segment

6 S T J depression 0 5—0 9 mm with normal configuration of S T segment
7 S T segment elevation 1 0 mm or more (I, II III, aVL aVF, CR_4 or CR_1)
T wave items (when R amplitude = 5 mm or more in aVL and QRS mainly upright in aVF)
V 1 T amplitude = minus 5 mm or more (I II CR_4—CR_1)
2 T amplitude minus 1 to 5 mm (I, II CR_4—CR_1)
3 T wave flat or small diphasic negative phase less than 1 mm (I II CR_4—CR_1)
A V conduction
VI 3 P R interval over 0 21 sec (any heart rate) (I II III)
4 Accelerated conduction (WPW)
Ventricular conduction
VII 1 Left bundle branch block, QRS duration *0 12* sec or greater in I II or III *and* R peak duration 0 06 sec or more in any of I II aVL CR_4 CR
2 Complete right bundle branch block, QRS duration 0 12 sec or greater *in I II III and* R prime greater than R in CR_1
3 Incomplete right bundle branch block R *prime greater* than R *and* QRS duration less than 0 12 sec in CP_1
5 Intraventricular block QRS 0 12 sec or more *and* no LBBB or RBBB (I II or III)
Arrhythmias
VIII 3 Atrial fibrillation
6 A V nodal rhythm (up to 100 *min*)
7 Sinus tachycardia (over *100 min*)
8 Sinus bradycardia under 50 *min*)
Arrhythmias Addition to the original code)
IX 1 Bigeminal trigeminal or quadrigeminal ventricular ectopic beats (VES)
2 Frequent 4 or more in 40 complexes) VES and supraventricular ectopic beats (SVES
3 Frequent VES
4 Frequent SVES
5 Occasional ES (less than 4 in 40 compl) VES and SVES
6 Occasional VES
7 Occasional SVES or ectopic atrial rhythm
Miscellaneous
X (IX in the original code)
2 Qualitative T wave findings including high or peaked T, post extrasystolic T wave inversion T notching etc
3 QRS findings not mentioned above including notching slurring PR prime rotation or others
5 P wave findings including peaked negative 3 mm amplitude or over or others
7 Other items not mentioned above

The classification of the complete ECG series in connection with exercise as a unit (exercise ECG) was with regard to the S T segment and T wave changes based on the ECG recorded 3 min after exercise (11) Arrhythmias and other ECG changes were coded whenever they occurred during or after exercise and the most severe of the changes were reported

Classification of S T changes Results and comments

There is general agreement on the prognostic importance of horizontal S T depressions (1 14 16) Opinion is divided however on the importance of strictly junctional changes (13 17)

For several years the importance has been pointed out of the difference between these two types of S T changes In too few studies however has the distinction actually been drawn Separate classification are necessary for valid comparison between them

At the high heart rates attained in the present study however it was difficult to distinguish between strictly junctional depressions (IV 5 6) and horizontal S T depressions (IV 1—4) This was shown by testing the reliability of the interpretation One observer coded with regard to the S T changes all the ECGs recorded at rest during and after exercise on two occasions

load was increased by steps with an exercise time of about 6 min at each level Most of the women began with a load of 300 kpm/min (oxygen uptake about 0,9 l/min), and the men at 600 kpm/min (oxygen uptake about 1,5 l/min) At the completion of each 6 minute period, the load was increased by 150 kpm/min for the women, and by 300 kpm/min for the men until a heart rate of about 150/min had been reached, provided that no symptoms or ECG changes caused an earlier termination of the test

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5 S T J depression 1 mm or more with normal configuration of S T segment

Table I Cont

1 min after work	2nd reading							
1st reading	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	59	23	14	1	20	41	46
IV 0	67	*54*				2		11
IV 1	17		*13*	1			3	
IV 2	12		5	*6*			1	
IV 3	–							
IV 4	18			1	1	*15*		1
IV 5	40	1	4	1			*27*	7
IV 6	50	4	1	5		3	10	*27*

3 min after work	2nd reading							
1st reading	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	79	22	21	1	36	6	39
IV 0	69	*66*						3
IV 1	19		*17*	2				
IV 2	21		5	*14*		2		
IV 3	3			1	*1*	1		
IV 4	46	7		4		*31*	1	3
IV 5	11	1					*4*	6
IV 6	35	5				2	1	*27*

with two years interval The results of the comparison of the two coding occasions are given in table I The agreement between them was very good with regard to the more severe changes (IV 1 2 3) at rest and 3 min after exercise Borderline changes however were for instance coded as IV 1 the first time and as IV 2 the second time which circumstance has no importance from a diagnostic point of view The incidence of significant S T changes in the group was about the same on both occasions These results agree well with similar ones of Blackburn (4) The agreement for the S T change of type IV 4 was somewhat worse but still satisfying The agreement for the changes recorded during immediately after and one min after work was not as good as for those recorded at rest and 3 min after work This was especially striking with regard to the separation of the two types of changes and most critical is the classification of type IV 6 It is difficult to say whether there is a biological basis for a combination of the two types of S T changes or whether such a combination is a result of interpretation difficulties At lower heart rates there arose however no difficulty in distinguishing between strictly junctional depressions and the segmental changes

It may be worthwhile to investigate the isolated J-depression (IV 5—6) as a possible fore runner of segmental depressions (IV 1—4) In the present study there was no tendency toward more frequent development of S T segment depressions in subjects who had previously had isolated J-depressions compared with those exhibiting no changes

TABLE I Intra observer reliability in coding S T depressions Repeated readings 2 years apart by one observer of ECG s (S T depr) at rest, during work, immediately after work 1 min after and 3 min after, in 204 men and women The table should be read in the following manner at the first reading, *3 min after work, there were for instance 46 ECG s coded* as IV 4 of these 31 got the same code in the second reading 7 got IV 0, 4 got IV 2 1 got IV 5 and 3 got IV 6

At rest, 1st reading	2nd reading							
	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	143	3	9	3	36	1	9
IV 0	132	*128*				2		2
IV 1	6		*3*	3				
IV 2	8			*6*	2			
IV 3	1				*1*			
IV 4	39	5				*34*		
IV 5	2						*1*	1
IV 6	16	10						*6*

During work, 1st reading	2nd reading							
	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	72	38	2	—	—	80	12
IV 0	78	*59*					12	7
IV 1	28		*25*	1			2	
IV 2	5		2	*1*			2	
IV 3	—							
IV 4	2	1						1
IV 5	77	5	11				*61*	
IV 6	14	7					3	*4*

Immediately after work, 1st reading	2nd reading							
	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	*81*	*38*	*2*	—	—	*71*	*12*
IV 0	85	*64*					14	7
IV 1	27		*25*	1			1	
IV 2	4		2	*1*			1	
IV 3	—							
IV 4	2	1						1
IV 5	71	10	11				*50*	
IV 6	15	6					5	*4*

Table 1 Cont.

1st reading	1 min after work	2nd reading							
		No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
	No	204	59	23	14	1	20	41	46
	IV 0	67	*54*				2		11
	IV 1	17		*13*	1			3	
	IV 2	12		5	*6*			1	
	IV 3	–							
	IV 4	18			1	1	15		1
	IV 5	40	1	4	1			*27*	7
	IV 6	50	4	1	5		3	10	*27*

1st reading	3 min after work	2nd reading							
		No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
	No	204	79	22	21	1	36	6	39
	IV 0	69	*66*						3
	IV 1	19		*17*	2				
	IV 2	21		5	*14*		2		
	IV 3	3			1	*1*	1		
	IV 4	46	7		4		*31*	1	3
	IV 5	11	1					*4*	6
	IV 6	35	5				2	1	*27*

with two years interval. The results of the comparison of the two coding occasions are given in table I. The agreement between them was very good with regard to the more severe changes (IV 1, 2, 3) at rest and 3 min after exercise. Borderline changes however were for instance coded as IV 1 the first time and as IV 2 the second time which circumstance has no importance from a diagnostic point of view. The incidence of significant S-T changes in the group was about the same on both occasions. These results agree well with similar ones of Blackburn (4). The agreement for the S-T change of type IV 4 was somewhat worse but still satisfying. The agreement for the changes recorded during immediately after and one min after work was not as good as for those recorded at rest and 3 min after work. This was especially striking with regard to the separation of the two types of changes and most critical is the classification of type IV 6. It is difficult to say whether there is a biological basis for a combination of the two types of S-T changes or whether such a combination is a result of interpretation difficulties. At lower heart rates there arose however no difficulty in distinguishing between strictly junctional depressions and the segmental changes.

It may be worthwhile to investigate the isolated J-depression (IV 5—6) as a possible fore-runner of segmental depressions (IV 1—4). In the present study there was no tendency toward more frequent development of S-T segment depressions in subjects who had previously had isolated J-depressions compared with those exhibiting no changes

TABLE I Intra observer reliability in coding S T depressions Repeated readings 2 years apart by one observer of ECG s (S T depr) at rest, during work, immediately after work 1 min after and 3 min after, in 204 men and women The table should be read in the following manner at the first reading, 3 min after work, there were for instance 46 ECG s coded as IV 4 of these 31 got the same code in the second reading 7 got IV 0, 4 got IV 2 1 got IV 5 and 3 got IV 6

At rest, 1st reading	2nd reading							
	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	143	3	9	3	36	1	9
IV 0	132	*128*				2		2
IV 1	6		*3*	3				
IV 2	8			*6*	2			
IV 3	1				*1*			
IV 4	39	5				*34*		
IV 5	2						*1*	1
IV 6	16	10						*6*

During work, 1st reading	2nd reading							
	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	72	38	2	—	—	80	12
IV 0	78	*59*					12	7
IV 1	28		*25*	1			2	
IV 2	5		2	*1*			2	
IV 3	—							
IV 4	2	1						1
IV 5	77	5	11				*61*	
IV 6	14	7					3	*4*

Immediately after work, 1st reading	2nd reading							
	No	IV 0	IV 1	IV 2	IV 3	IV 4	IV 5	IV 6
No	204	81	38	2	—	—	71	12
IV 0	85	*64*					14	7
IV 1	27		*25*	1			1	
IV 2	4		2	*1*			1	
IV 3	—							
IV 4	2	1						1
IV 5	71	10	11				*50*	
IV 6	15	6					5	*4*

TABLE III The frequency of ECG changes codes I II III VI VII and X in 204 females and males in 1954 and 1962 The table gives the number of coded items not the number of subjects (applies also to tables IV and V)

		1954				1962			
		Resting ECG		Exercise ECG		Resting ECG		Exercise ECG	
		♀	♂	♀	♂	♀	♂	♀	♂
No of subjects		117	87	117	87	117	87	117	87
I	0	66	51	48	65	46	45	21	31
I	2						1		
II	1	3	3			3	1		
III	1	2	2			4	3		
VI	3	2	1			1			
	4	1		1		1		1	
VII	2			1				1	
	3	1		1		1		1	
	4	1		1		1		1	
X	2	1	4				1		
	3	11	9			11	7		
	5	2	2			1			
	7						1		

There was one subject (male) in the entire group who had had one myocardial infarction (I II) between 1954 and 1962 In 1954 his resting and exercise ECGs were both normal The number of subjects with left axis deviation AV block I pre excitation syndrome intraventricular conduction defects and minor changes is given in table III There were 3 subjects with high R amplitudes in leads CR_4 or CR_5 III 1 in both 1954 and 1962 (one in 1954 only) and 4 who had developed this change by 1962 Of these 7 subjects there were 6 with S T segment changes IV I—4 at *rest and or* 3 min after exercise in 1962 and 5 with elevated blood pressure

SUBJECTS WITH ELEVATED BLOOD PRESSURE

a S T T changes codes IV and V

Of 204 subjects 35 (21 women and 14 men) had elevated blood pressure in 1962 (≥ 165 and/or ≥ 95 mm Hg) Of these 35 8 had no or only minor ECG changes on both examinations Twelve subjects had type IV 1—4 S T changes at rest in 1954 In 1962 the number was 18 In 1954 16 subjects had S T segment depressions 3 min after exercise and in 1962 the number was 22 (table IV) Of these 22 8 had progressed 6 had developed during the follow up period 6 were unchanged and 2 had become less marked in 1962 than in 1954 Of the 6 subjects with an unchanged

TABLE II The frequency of strictly junctional depressions code IV 5, 6 in females and males 1954 and 1962

			1954					1962				
			No	At rest	During exercise	1 min after	3 min after	No	At rest	During exercise	1 min after	3 min after
♀	IV	5	117	3	32	18	4	117	1	44	17	3
		6	117	2	2	20	9	117	6	11	36	19
♂	IV	5	87	—	19	10	5	87	1	33	23	8
		6	87	1	2	6	4	87	10	3	14	16

(There were 42 subjects who had S T changes of type IV 5 during exercise, or IV 5 or 6 after exercise in the 1954 data These, however, were without type IV 1—4 changes 3 min after exercise Nine of these, that is 21 %, had acquired type IV 1—4 changes 3 min after exercise in 1962 This frequency should be compared with the number of subjects who had no S T changes of any sort in 1954 but who, in 1962, had acquired type IV 1—4 changes 3 min after exercise In 1954 the number of subjects without S T changes was 96, of whom 22 (23 %) had developed segmental depressions in 1962)

During exercise and 1 min after exercise strictly junctional depressions of 0 5 mm or more occurred in somewhat less than 50 % of both women and men (table II) Three min after exercise the corresponding frequency had declined to 20—25 % These results agree well with those reported by Rumball and Acheson (17) After exercise S T segment depressions (type IV 1—4) increased from 17 % to 44 % from the 1st to the 3rd min Thus with a decrease in heart rate the number of strictly junctional depressions decreased while the number of S T segment depressions increased The same observation has been made by Rumball and Acheson (17), Mattingly (14) and Lepeschkin and Surawics (12) This would imply that strictly junctional depressions after exercise (at least if they are less than 2 mm) are of a functional nature

Results

ELECTROCARDIOGRAMS

Q and QS changes, QRS-axis deviations, high R amplitudes, atrio-ventricular and intraventricular conduction defects, and minor changes, codes I, II, III, VI, VII and X

The frequency of ECG changes at rest and during and after exercise in 204 women and men in 1954 and 1962 is given in table III Sixty-six of 117 women (56 %) and 51 of 87 men (59 %) had normal resting ECGs in 1954 (no reportable changes according to the code used) The corresponding figures for 1962 were 46 (39 %) and 45 (52%), respectively During and after exercise, 48 women (41 %) and 65 men (75 %) had normal ECGs in 1954 In 1962 the corresponding figures were 21 women (18 %) and 31 men (36 %) (Changes classified as IV 5 or 6 *during* work have been considered normal)

TABLE III The frequency of ECG changes codes I II III VI VII and X, in 204 females and males in 1954 and 1962 The table gives the number of coded items not the number of subjects (applies also to tables IV and V)

		1954				1962			
		Resting ECG		Exercise ECG		Resting ECG		Exercise ECG	
		♀	♂	♀	♂	♀	♂	♀	♂
No of subjects		117	87	117	87	117	87	117	87
I	0	66	51	48	65	46	45	21	31
I	2						1		
II	1	3	3			3	1		
III	1	2	2			4	3		
VI	3	2	1			1			
	4	1		1		1		1	
VII	2			1				1	
	3	1		1		1		1	
	4	1		1		1		1	
X	2	1	4				1		
	3	11	9			11	7		
	5	2	2			1			
	7						1		

There was one subject (male) in the entire group who had had one myocardial infarction (I II) between 1954 and 1962 In 1954 his resting and exercise ECGs were both normal The number of subjects with left axis deviation A V block I pre-excitation syndrome intraventricular conduction defects and minor changes is given in table III There were 3 subjects with high R amplitudes in leads CR_4 or CR_5 (III I) in both 1954 and 1962 (one in 1954 only) and 4 who had developed this change by 1962 Of these 7 subjects, there were 6 with S T segment changes IV 1—V at rest and or 3 *min* after exercise in 1962 and 5 with elevated blood pressure

SUBJECTS WITH ELEVATED BLOOD PRESSURE

a S T T changes codes IV and V

Of 204 subjects 35 (21 women and 14 men) had elevated blood pressure in 1962 (> 165 and or > 95 mm Hg) Of these 35 8 had no or only minor ECG changes on both examinations Twelve subjects had type IV 1—4 S T changes at rest in 1954 In 1962 the number was 18 In 1954 16 subjects had S T segment depressions 3 min after exercise and in 1962 the number was 22 (table IV) Of these 22 8 had progressed 6 had developed during the follow up period 6 were unchanged and 2 had become less marked in 1962 than in 1954 Of the 6 subjects with an unchanged

TABLE IV The frequency of ECG changes in 1954 and 1962, coded as IV, V and IX, in 35 females and males with an elevated resting blood pressure in 1962

		1954				1962			
		Resting ECG		Exercise ECG		Resting ECG		Exercise ECG	
		♀	♂	♀	♂	♀	♂	♀	♂
No of subjects		21	14	21	14	21	14	21	14
IV	1	3		4	1	4		6	1
	2	1	1	4		3	1	5	2
	3							1	
	4	7		7		8	2	4	3
V	2	1		1					1
	3	6	2	5	2	9	5	8	2
IX	3		[1]1						
	4			1					
	6				[1]1			1	
	7		1		1			2	1

[1] Same subject

picture, there were 2 (females) with serious S-T depression on both occasions (IV 1). One subject had S-T depression at rest (IV 4), which disappeared after exercise (1954).

The number of subjects with T wave changes (V 2—3) is given in table IV. Only one of these subjects lacked S-T changes (IV 1—4). A marked T wave change always occurred in conjunction with a marked S-T change.

b Arrhythmias, code IX

There were 7 subjects with premature beats (PMB) of various types. None of these was present in both 1954 and 1962. There were 4 cases of premature beats combined with S-T changes (IV 1—4).

NORMOTENSIVE SUBJECTS

a S-T-T changes, codes IV and V

Of 169 subjects (204—35), 96 women and 73 men, with a resting blood pressure below 170 and 100 mm Hg, there were 18 (19 %) women and 2 (3 %) men with type IV 1—4 S-T changes at rest in 1954 (table V). The corresponding figures for 1962 were 31 (32 %) and 5 (7 %), respectively. In 1954 36 women and 5 men had the mentioned changes, IV 1—4, 3 min after exercise. The figures for 1962 were 49 and 18.

Two subjects in 1954 and 2 additional subjects in 1962 had type IV 4 S-T changes at rest, which disappeared or remained unchanged after exercise.

Of the 41 subjects who in 1954 had S-T changes 3 min after exercise,

TABLE V The frequency of ECG changes in 1954 and 1962 coded as IV, V, VIII and IX in 169 normotensive females and males

		1954				1962			
		Resting ECG		Exercise ECG		Resting ECG		Exercise ECG	
		♀	♂	♀	♂	♀	♂	♀	♂
No of subjects		96	73	96	73	96	73	96	73
IV	1	2		4	1	2		9	3
	2	2	1	2	3	2	2	9	5
	3	1		5		1		2	
	4	13	1	25	1	26	3	29	10
	7		3		3		1		
V	2	3		3		2	1	1	1
	3	5	4	11	2	10	9	17	9
VIII	6	1				1		1	
	7	2	3			1	2		
	8	1				1	1		
IX	1								1
	2		1						
	3				1			1	
	4			1		1	3	4	4
	5							1	
	6		3	2	2	1	2	11	4
	7	1	1	4	2	2	1	8	9

there were 6, all with the minimal changes (IV 4), who in 1962 had no S-T deviation 3 min after exercise. Eighteen remained unchanged, 2 of whom had marked changes in both 1954 and 1962. 15 had deteriorated and 2 had lesser changes in 1962 than in 1954. There were 33 subjects with newly developed S-T changes either at rest or 3 min after exercise in 1962.

Of the 41 type IV 1—4 S-T changes 3 min after exercise in 1954 there were 23 with no changes at rest in the same year. In 1962 9 of these 23 subjects had acquired changes at rest while 10 retained changes after exercise only and 4 exhibited no change either at rest or after exercise.

Of the above mentioned 41 subjects with S-T changes 3 min after exercise in 1954, 26 were in the minimal change category (IV 4). In 1962, as formerly noted, 6 of these 26 exhibited no S-T changes 3 min after exercise. Eleven remained unchanged and 9 had become more marked.

The number of subjects with T changes (V 2—3) is given in table V. Only 1 subject with T wave changes (V 2) did not also have S-T changes (IV 1—4).

TABLE VI Symptoms during the bicycle exercise in 1962

	♀		♂	
	Normal resting B P	Elevated resting B P	Normal resting B P	Elevated resting B P
1	20	8	22	5
2	3	—	2	—
3	41	5	28	4
4	6	1	2	—
5	26	6	19	4
6	—	1	—	1
Total	96	21	73	14

1 = dyspnea, 2 = chest pain, 3 = joint and muscle pain 4 = no complaints, 5 = other complaints 6 = 1 and 2 combined

Minor T changes (V 3) without concommitant S-T changes (IV 1—4) occurred more often

b Arrhythmias, codes VIII and IX

Table V gives the number of subjects with A-V nodal rhythm, sinus tachycardia and sinus bradycardia

At rest 6 subjects (1 female, 5 males) had premature beats (PMB) in 1954 The 1962 frequency was 10 (4 females, 6 males) During and after exercise 12 subjects (7 females, 5 males) developed PMB in 1954 and 37 (22 females, 15 males) in 1962

There was no significant difference between females and males in this respect The total number of subjects with PMB in 1954 was 15 and in 1962 it was 39 There was a total of 47 (28 %) subjects with PMB on one or more occasions Fourty of these 47 exhibited PMB in only one of the trials, either at rest and/or in connection with exercise Of the 7 subjects with PMB on both testing occasions, 2 remained unchanged from 1954 to 1962 The remaining 5 varied between a single and multiple supraventricular and/or ventricular PMB from 1954 to 1962

In 1962 PMB (IX 6—7) combined with S-T changes (IV 4) occurred in only 2 subjects at rest Of the 12 subjects with PMB during or after exercise in 1954, there were 7 with S-T changes, 5 of whom were of type IV 4 Of the 37 in 1962, there were 14 with S-T changes, 9 of whom were of type IV 4 The probability of PMB and horizontal S T depressions occurring concommitantly during or after exercise was estimated by the χ^2 test (P = 0,5 in 1954 (uncertain correlation due to small n) and P < 0,001 in 1962) The difference in incidence between 1954 and 1962 was statistically significant

SYMPTOMS

The number of subjects with various types of symptoms during the bicycle exercise is given in table VI Thirteen of 35 subjects with elevated blood pressure

TABLE VII Mean ± 1 × SD heart rate at various loads and final heart rate for females and males in 1962

	No	300 kpm/min	No	450 kpm/min	No	600 kpm/min	No	900 kpm/min	No	Final heart rate
♀	106	123±16	88	138±17	32	146±13	—	—	117	141±17
♂	26	117±11	—	—	81	128±17	63	149±14	87	148±15

complained of dyspnea 2 of dyspnea combined with chest pain 9 of joint and muscle pain and the remainder reported either no distress or symptoms irrelevant to the testing situation Of 169 normotensive subjects, 42 complained of dyspnea, 5 of chest pain 69 of joint and muscle pain and the remainder had either no or irrelevant symptoms

Thus there was a total of 55 subjects with dyspnea during exercise Twenty three of these had type IV 1—4 S T changes and there were 9 with PMB (IX 4—7) Of the 7 subjects reporting chest pain 5 had type IV 1—4 S T changes Of the 78 subjects with joint and muscle pain 25 had type IV 1—4 S T changes and 17 had PMB (IX 4—7) Of the 64 subjects with illdefined symptoms of relevance 36 had type IV 1—4 S T changes and 15 had PMB (IX 4—7)

WORK LOAD AND HEART RATE

The mean heart rate at the termination of exercise was for women 151 beats/min in 1954 and 141 beats min in 1962 Corresponding values for the men were 154 in 1954 and 148 in 1962 The standard deviation was about ± 15 beats min for all these groups

The 1962 average heart rate for the women was 123 at 300 kpm min 138 at 450 kpm/min and 146 at 600 kpm/min (table VII) The average heart rate for the men was 117 at 300 kpm/min, 128 at 600 kpm/min and 149 at 900 kpm/min

Since there was no heart rate difference between the normotensives and those with an elevated resting blood pressure, all subjects were included in these calculations

Discussion

During the eight year interval between the two studies the frequency of subjects with marked S T changes (type IV 1 2 or 3) 3 min after exercise increased among females from 16 % to 27 % and among males from 6 % to 13 %

Rumball and Acheson (17) found in 360 RAF men the following frequencies age 35—39 years 8 % 40—44 years 14 % 45—49 years 14 % and 50—54 years 19 %

Among a sample of delivery personnel in Stockholm (3) consisting of 73 men between the ages of 50—65 years (x̄ = 54 years) the corresponding frequency 3 min after work (IV 1 2 or 3) was 18 % This had increased to 37 % 5 years later These figures agree well with Strandell's data (18) but a direct

comparison is not possible since he did not use the same criteria

Lepeschkin and Suravics (12) found among volunteer female students a frequency of 29 % in 45 women of 30—45 years, 23 % in 40 women of 46—55 years and 53% in 19 women over 55 years of age

Although all these individuals were selected and cannot be said to represent any one population group, the results of the studies agree quite well As judged from all these groups, the frequency of type IV 1—3 S-T changes should be less than 10 % in men below 40 years of age about 15 % at 40—50, 20 % at 55 years and about 35 % at 60 years of age For women the rate at 40—45 years should be about 20 %, at 50—55 years 30 % and over 55 years about 50 %

With regards to females, there are actually few data available It is notable that in the Framingham study (7), a higher frequency of angina pectoris was found among females than among males, while mortality and morbidity from myocardial infarction showed the expected preponderance among males *Gubner (9) does not consider type 1—4* S-T changes specific for ischemia among older subjects and particularly not among females over 40 years of age, since a decreased potassium content of the myocardium with increasing age may play an important role in its appearance

It is natural that the minimal change, IV 4, more often occurs sporadically than do the more marked changes It is important nevertheless that it be observed and noted, since it may be the forerunner of a more definite change The results of the current study and of that done in 1963 are both in favor of this conclusion

The frequency of PMB recorded during and after exercise rose from 18 in 1954 (9 %) to 43 in 1962 (21 %) In the material reported earlier by Åstrand (3) the frequency for males between the ages of 50—65 years was 45 %, while 5 years later the frequency had increased to 47 % This agrees well with the data reported by Strandell (18) The frequency of both S-T depressions (IV 1—4) and PMB increases with age, but in the three Swedish studies cited, comprising about 400 subjects, they were in most age groups not intercorrelated

*The heart rate at 300 and 450 kpm/*min for women agrees with results reported earlier by Åstrand (2) At 600 kpm/min the heart rate was lower, probably due to the fact that only subjects with a relatively large aerobic capacity could perform at this load For men, the results agree at 600 and 900 kpm/min with those reported previously (2, 19)

Summary

Twenty-one females and 14 males with an elevated resting blood pressure and 96 females and 73 males with a normal resting blood pressure between the ages of 48—63 years were examined twice with an interval of 8 years ECG s were recorded both at rest and during and after exercise on a bicycle ergometer The ECGs were classified according to a modified Minnesota code '

The frequency of S-T depressions and premature beats increased from the first to the second examination The frequency in various age groups is discussed

Segmental S T changes of the minimal type e g a change of form in the segment without any measurable depression, observed at the first examination progressed often into a more marked type at the second one Consequently, this type should be notified

The frequency of subjects with an initially completely normal ECG, who exhibited segmental changes 3 min after exercise 8 years later was about 20 % The frequency of subjects with a strictly junctional depression during or after exercise at the first examination who 8 years later had developed segmental S T depression was also about 20 %

The importance of distinguishing between these two types of S T changes is stressed S-T segment changes occurred independently of premature beats

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Blood Pressure During Physical Work in a Group of 221 Women and Men 48—63 Years Old

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In assessing borderline values for resting blood pressure the question often arises as to whether or not the pressure is elevated in the measurement situation only If the blood pressure reaction is normal in connection with physical work one ought to be able to dismiss the diagnosis of *hypertension, at least temporarily* On the other hand some persons with a normal resting blood pressure react with a marked blood pressure elevation during physical work Can an early diagnosis of hypertension be arrived at on the basis of such a reaction? The present study mainly concerns the feasibility of avoiding a false positive diagnosis of hypertension by blood pressure measurements during and after work as well as at rest *In this connection* blood pressure was measured before during and after work on a bicycle ergometer in a group of 221 men and women between the ages of 48—63 years

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Material and methods

Participating in the study were subjects selected for the Stockholm *City Health* Survey 1962 This 1962 study was a follow up of a sample population first examined in 1954 *For details about the material the reader is* referred to Frisk et al (5) and Åstrand (2) 197 persons participated out of the 204 in the *sub-sample previously reported by Åstrand* Twenty four additional subjects were included in the present study This latter group was composed of subjects selected for the *Health Study whose data from the year 1954 had* not been included in the analysis for technical reasons The entire group consisted of 103 women and 80 men with normal resting blood *pressure* ($<$ 170 *systolic and* $<$ 100 *mm* Hg diastolic pressure) and 22 women and 16 men with elevated pressure The mean age was 54 years for both men and women

The methodology used in the exercise test was described in a previous article (2) Indirect blood pressure measurements were taken during work at varying intensities in a sitting position on the bicycle ergometer Measurements were taken at the 5th min of each work level and 3 min after work in the

TABLE I Means ± 1 SD for blood pressure at rest, during and after exercise for women and men with normal and elevated resting blood pressure

		No		Rest	During exercise at heart rate M ± 1 SD			3 min after exercise
					109±9	130±5	148±5	
♀	Normal resting B P	103	Systolic	134±15	170±19	188±20	196±22	142±14
			Diastolic	85±8	89±8	93±11	96±13	83±2
♀	Elevated resting B P	22	Systolic	179±22	206±15	219±10	228±18	181±22
			Diastolic	107±15	112±13	113±12	115±20	102±10
					During exercise at heart rate M ± 1 SD			
					110±5	129±6	150±6	
♂	Normal resting B P	80	Systolic	137±15	180±24	199±20	212±23	147±14
			Diastolic	84±9	92±11	96±12	96±13	85±9
♂	Elevated resting B P	16	Systolic	166±16	212±28	225±26	242±33	178±22
			Diastolic	106±12	116±23	116±15	117±14	101±10

supine position The cuff was fastened around the left arm with an adhesive nylon hook surface The cuff contained a rubber bulb measuring 12 5 × 24 cm All measurements were performed by the same observer with a mercury manometer The diastolic pressure was recorded at the point where the Korotkow sounds changed in character (4th phase)

Results

Blood pressure at rest

The systolic pressure measured at rest in a supine position averaged 134 mm Hg for the 103 women The diastolic mean for the same group was 85 mm (table I) For the 80 men the corresponding values were 137 mm Hg and 84 mm Hg

The 22 women with an elevated resting blood pressure had a mean systolic pressure of 179 mm and a diastolic pressure average of 107 mm Hg The corresponding values for the 16 men were 166 and 106 mm Hg

Blood pressure during work

The pressures measured during work were arranged within 5 groups according to heart rate 1) ≤ 99 beats/min , 2) 100—119, 3) 120—139 4) 140—159 5) > 160 beats/min The mean heart rates and systolic and diastolic blood pressures were determined for each group (fig 1) In the heart-rate range between 100 and 159, there seemed to be a linear relationship between average blood pressures and heart rates Blood pressures at heart rates ≤ 99 beats/min were higher and at heart rates > 160 beats/min were lower than expected for the men This is probably due to a skewed distribution

of the material Consequently, all values measured at a heart rate $\leqslant 99$, and $\geqslant 160$ beats/min were eliminated from the subsequent analysis Using this criterion of exclusion, the number of observations per subject was reduced to 1 or 2 The regression lines were thereafter calculated for the relationship between systolic and diastolic blood pressure and pulse rate for women and men with a normal resting blood pressure The number of measurements and other data involved in these regression lines are given in table II The regression lines are to be found in fig 1, with the previously mentioned means also included Data for those with elevated resting blood pressure are given in table I

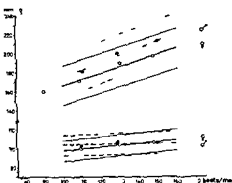


Fig 1 Systolic and diastolic blood pressure in relation to heart rate during exercise for women (————) and for men (— — —) with normal resting blood pressure The thin lines represent ± 1 SD Statistical data are given in table II The average systolic and diastolic blood pressure for each heart rate group is also given (○ = women ● = men)

Blood pressure 3 min after work

The 103 women with a normal resting blood pressure had 3 min after work an average *systolic blood pressure of 142* mm Hg, and an average diastolic pressure of 83 mm Hg (table I) The corresponding values *for the 80 men were 147* and 85 mm Hg Thus the systolic pressure was about 10 mm higher 3 min after *work than before, while the diastolic re*mained unchanged

TABLE II *Data from the regression calculation on the relationship between systolic and diastolic* blood pressure and heart rate during exercise in women and men with normal resting blood pressure The numerical value for r is in all cases stastically significantly different from 0 (P< 0 001) The numerical values for b (slope of the line) for women and men are not significantly different

		No	$\bar{x} \pm \varepsilon\bar{x}$	$\bar{y} \pm \varepsilon\bar{y}$	Regr eq	Corr coeff	Deviation fr regr line
Systolic B P in relation to heart rate	♀	169	130±1 1	186±1 8	y = 100 8 + 0 65x	r = 0 44±0 06	±21
Diastolic B P in relation to heart rate	♀	169	130±1 1	93±0 9	y = 70 0 + 0 18x	r = 0 25±0 07	±11
Systolic B P in relation to heart rate	♂	124	131±1 6	198±2 3	y = 101 1 + 0 74x	r = 0 49±0 07	±22
Diastolic B P in relation to heart rate	♂	124	131±1 6	95±1 1	y = 89 8 + 0 04x	r = 0 59±0 06	±12

TABLE I Means ± 1 SD for blood pressure at rest, during and after exercise for women and men with normal and elevated resting blood pressure

		No		Rest	During exercise at heart rate M ± 1 SD			3 min after exercise
					109±9	130±5	148±5	
♀	Normal resting B P	103	Systolic	134±15	170±19	188±20	196±22	142±14
			Diastolic	85±8	89±8	93±11	96±13	83±2
♀	Elevated resting B P	22	Systolic	179±22	206±15	219±10	228±18	181±22
			Diastolic	107±15	112±13	113±12	115±20	102±10
					During exercise at heart rate M ± 1 SD			
					110±5	129±6	150±6	
♂	Normal resting B P	80	Systolic	137±15	180±24	199±20	212±23	147±14
			Diastolic	84±9	92±11	96±12	96±13	85±9
♂	Elevated resting B P	16	Systolic	166±16	212±28	225±26	242±33	178±22
			Diastolic	106±12	116±23	116±15	117±14	101±10

supine position The cuff was fastened around the left arm with an adhesive nylon hook surface The cuff contained a rubber bulb measuring 12 5 × 24 cm All measurements were performed by the same observer with a mercury manometer The diastolic pressure was recorded at the point where the Korotkow sounds changed in character (4th phase)

Results

Blood pressure at rest

The systolic pressure measured at rest in a supine position averaged 134 mm Hg for the 103 women The diastolic mean for the same group was 85 mm (table I) For the 80 men the corresponding values were 137 mm Hg and 84 mm Hg

The 22 women with an elevated resting blood pressure had a mean systolic pressure of 179 mm, and a diastolic pressure average of 107 mm Hg The corresponding values for the 16 men were 166 and 106 mm Hg

Blood pressure during work

The pressures measured during work were arranged within 5 groups according to heart rate 1) ≤ 99 beats/min , 2) 100—119, 3) 120—139, 4) 140—159, 5) ≥ 160 beats/min The mean heart rates and systolic and diastolic blood pressures were determined for each group (fig 1) In the heart rate range between 100 and 159, there seemed to be a linear relationship between average blood pressures and heart rates Blood pressures at heart rates ≤ 99 beats/min were higher and at heart rates > 160 beats/min were lower than expected for the men This is probably due to a skewed distribution

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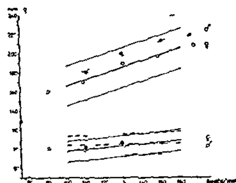


Fig 1 Systolic and diastolic blood pressure in relation to heart rate during exercise for women (———) and for men (— — —) with normal resting blood pressure The thin lines represent ± 1 SD Statistical data are given in table II The average systolic and diastolic blood pressure for each heart rate group is also given (○ = women ● = men)

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The 103 women with a normal resting blood pressure had, 3 min after work, an average systolic blood pressure of 142 mm Hg and an average diastolic pressure of 83 mm Hg (table I) The corresponding values for the 80 men were 147 and 85 mm Hg Thus the systolic pressure was about 10 mm higher 3 min after work than before while the diastolic remained unchanged

TABLE II Data from the regression calculation on the relationship between systolic and diastolic blood pressure and heart rate during exercise in women and men with normal resting blood pressure The numerical value for r is in all cases stastically significantly different from 0 ($P < 0.001$) The numerical values for b (slope of the line) for women and men are not significantly different

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Diastolic B P in relation to heart rate	♂ 124	131 ± 1 6	95 ± 1 1	y = 89 8 + 0 04x	r = 0 59 ± 0 06	± 12

The 22 women with an elevated resting blood pressure had an average systolic blood pressure of 181 mm Hg, and an average diastolic of 102 mm Hg 3 min after work. The corresponding figures for the men were 178 and 101 mm Hg. Thus the average diastolic blood pressure was lower 3 min. after work than before work, while the systolic remained the same or showed a rise.

ECG changes

Detailed results of ECGs taken at rest and in connection with exercise are given in a previous paper (2), which contains ECG findings for 35 of the present 38 subjects with an elevated resting blood pressure. Of the remaining 3 subjects, 1 woman had a horizontal (Type IV: 1) S-T depression of 1 mm or more both at rest and 3 min. after work, 2 men had ischemic type S-T depressions of 0.5—0.9 mm 3 min. after work. Altogether 25 out of 38 subjects (65 %) had significant S-T segment changes in connection with work (77 % of the women and 50 % of the men). Of those with a normal blood pressure reaction both at rest and at work, 49 women (51 %) had horizontal S-T changes in connection with work. The corresponding frequency for the men was 18 (25 %).

Of the 38 subjects with an elevated blood pressure at rest, 8 showed an exercise blood pressure reaction within the regression line $\pm 1 \times \sigma$. Of these 8, 7 showed either no or insignificant ECG changes (Code IV: 5, 6). In this group of 38 subjects there were 6 additional persons who had unimportant ECG changes, while the rest had more serious changes. Nine of the 183 "normal" subjects had an exercise blood pressure reaction beyond the regression line $+ 2 \times \sigma$. Two of these 9 had S-T segment changes, while the rest were normal.

Of those with an elevated resting blood pressure, 5 had a normal pressure 3 min. after work. Two of these 5 had also an exercise blood pressure within the regression line $\pm 1 \times \sigma$. In other words, 2 of the above-mentioned 8 subjects had an elevated resting blood pressure, but a normal pressure during, as well as after, work. The remaining 3 subjects of the 5 with a normal pressure after work exhibited an elevated pressure during work which reverted to normal after work. Of these 3 subjects 2 had S-T segment changes (Type IV: 2), and 1 had a normal ECG reaction.

Discussion

General viewpoints on ECG changes in arterial hypertension are given by Lord and Hellerstein (4). It is probable that the 7 subjects in the present study who had an elevated resting blood pressure, but a normal pressure reaction during work, and no ECG changes of any significance, should be considered normotensive.

The resting blood pressures found in this study agree with Tibblin et al.'s data on 50 year old men (sitting) from Gothenburg (9).

At an exercise heart rate of 128, Granath et al. (6) found an intra-arterial systolic pressure of 194 mm and a diastolic of 82 mm in 9 men between the ages of 61—83 years. In the present material, at a heart rate of 130 beats/min the men had a systolic pressure of 197

mm and a diastolic of 95 mm Thus the indirect systolic pressure values in the present study agree well with Granath et al s intra arterial pressures in a group 10—15 years older (for references on the comparison of intra arterial with indirect measurements see Holmgren (7) The diastolic values, however, are approximately 10 mm higher This difference is however not only found during work but also at resting conditions (8)

In order to arrive at meaningful comparisons between various age groups the measurements must be taken at the same relative loads With an age difference of 10—15 years the difference in the relative load at a given heart rate is rather small, but must be considered with greater age differences Holmgren (7) found in a group of 35 men in their 20 s at a heart rate of 160 beats/min, an intra arterial systolic pressure of 170 mm and a diastolic pressure of 83 mm This heart rate of 160 in a group of 20 years old subjects corresponds to about the same relative load as a heart rate of 130 in a group of 60 years old subjects (1) Thus there remains a definite age difference in systolic blood pressure in relation to heart rate even if the comparison is made at the same relative load

At a given heart rate women normally employ a smaller proportion of their aerobic capacity than men do (3) Provided that both men and women at a given age attain the same maximal exercise blood pressure the women s blood pressure ought to be lower at a given heart rate than the men s According to this reasoning the women ought to be comparable with men at a heart rate 10—15 beats min faster Using such a correction factor in the present study the same pressure reaction was found in men as in women

The deviation around the regression line for the relationship between blood pressure and heart rate in the present material is large for both men and women At a given heart rate different individuals do not employ the same proportion of their aerobic capacity This dissimilarity depends upon, among other factors varying maximal heart rates and probably explains part of this wide spread At 3—6 min of maximal physical work when the individual attains his maximal heart rate the spread ought to be diminished In other words the spread probably could have been reduced if the percentage of employed aerobic capacity, rather than the heart rate had been placed on the abscissa In spite of this scatter however an exercise test limited to heart rate and direct or indirect blood pressure measurements and ECG analysis is worthwhile in cases of suspected hypertension

Summary

Indirect blood pressures were measured at rest and during and after exercise on a bicycle ergometer in a normotensive group of 103 women and 80 men between the ages of 48—63 years This examination was done also on a group of 22 women and 16 men within the same age range with an elevated resting blood pressure The values agree in general with those from previous intra arterial measurements made on the same age group

The 22 women with an elevated resting blood pressure had an average systolic blood pressure of *181* mm *Hg* and an average diastolic of 102 mm Hg 3 min after work The corresponding figures for the men were 178 and 101 mm Hg Thus the average diastolic blood pressure was lower 3 min after work than before work while the systolic remained the same or showed a rise

ECG changes

Detailed results of ECGs taken at rest and in connection with exercise are given in a previous paper (2) which contains ECG findings for 35 of the present 38 subjects with an elevated resting blood pressure Of the remaining 3 subjects 1 woman had a horizontal (Type IV 1) S T depression of 1 mm or more both at rest and 3 min after work 2 men had ischemic type S T depressions of 0 5—0 9 mm 3 min after work Altogether 25 out of 38 subjects (65 %) had significant S T segment changes in connection with work (77 % of the women and 50 % of the men) Of those with a normal blood pressure reaction both at rest and at work 49 women (51 %) had horizontal S T changes in connection with work The corresponding frequency for the men was 18 (25 %)

Of the 38 subjects with an elevated blood pressure at rest 8 showed an exercise blood pressure reaction within the regression line $\pm$ 1 $\times$ σ Of these 8 7 showed either no or insignificant ECG changes (Code IV 5 6) In this group of 38 subjects there were 6 additional persons who had unimportant ECG changes while the rest had more serious changes Nine of the 183 normal subjects had an exercise blood pressure reaction beyond the regression line + 2 $\times$ σ Two of these 9 had S T segment changes while the rest were normal

Of those with an elevated resting blood pressure, 5 had a normal pressure 3 min after work Two of these 5 had also an exercise blood pressure within the regression line $\pm$ 1 $\times$ σ In other words 2 of the above mentioned 8 subjects had an elevated resting blood pressure but a normal pressure during as well as after work The remaining 3 subjects of the 5 with a normal pressure after work exhibited an elevated pressure during work which reverted to normal after work Of these 3 subjects 2 had S T segment changes (Type IV 2) and 1 had a normal ECG reaction

Discussion

General viewpoints on ECG changes in arterial hypertension are given by Ford and Hellerstein (4) It is probable that the 7 subjects in the present study who had an elevated resting blood pressure but a normal pressure reaction during work and no ECG changes of any significance should be considered normotensive

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From the University Lung Clinic (Head Gösta Birath M D), Renströmska sjukhuset, Göteborg Sweden

Idiopathic Interstitial Fibrosis of the Lungs

I Prognosis as Indicated by Radiological Findings

By

Lars Andér[1]

During the last 10 years the syndrome idiopathic interstitial fibrosis of the lungs (IFL) has been defined clinically, physiologically and anatomically (3, 6, 10 15 17) Livingstone et al (12) have recently given a detailed review of the literature

The clinically and anatomically acute syndrome which was described by Hamman and Rich in 1944 (8) is considered by most authors to be an acute form of IFL Scadding wishes to limit the name Hamman—Rich fibrosis to the acute fulminant form and to leave the name interstitial fibrosis of the lungs to the syndrome as follows A middle aged or elderly person who having previously been well complains of progressive dyspnoea on exertion over the preceding few years has a cough perhaps troublesome but usually unproductive may be cyanosed especially after exercise has gross clubbing of the fingers has rales at the bases of the lungs has no obvious cardiac abnormality and is not constitutionally ill can be suspected with some confidence of suffering of chronic diffuse interstitial fibrosis of the lungs' (17)

It is nowadays considered justifiable to diagnose interstitial fibrosis of the lungs by means of clinical history, symptomatology radiological and physiological findings and without obtaining biopsy of the lungs (7 12 16, 17)

In a series of papers data will be presented from 19 cases of interstitial fibrosis of the lungs, collected from the years 1952—1963 The present paper deals with course and duration of the disease, classification of the X ray findings and prognosis as related to X ray findings and to treatment with steroids

The other papers in this series deal with

II The reversibility of the respiratory disturbances after steroid administration (13)

III The pathology (2 a) and

IV Heredity studies (1)

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Women and men attained the same systolic and diastolic pressure at a given *relative* load, but attained a differing pressure at a given heart rate

Of the 38 subjects with an elevated resting blood pressure, 8 had a normal exercise pressure reaction Of these 8, 7 showed no or insignificant ECG changes, and probably had a *functionally elevated resting blood pressure*

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f rst at necropsy See table I and paper III (2 a)

The *cl n al history* was obtained from the rout ne case records For d agnos s IFL was requ red that the symptoms started at some def n te t me and that chron c productive or purulent bronch tis had not occurred

The t me for *onset of the d ease* as ind cated by the cl n cal history was confirmed by the rad olog cal find ngs This of course re qu red a check up of all earl er rout ne f lms and m crof lms

The rad ological techn que for *rad ograms* of the chest was standard zed as far as pos-s ble n general at 130—140 kv The low k lo olt techn que 70—80 kv was used n an attempt to detect early honeycomb pattern but d d not prove to be of any ad antage Tomograms and bronchograms were ob-ta ned in most cases

D a no t c tho acotomy was performed in most cases us ng the techn que described by Andér and Bergh (2) with inspect on and palpat on of the lung and b opsy from d seased and non-d seased parts of the lung The exc sed samples were stud ed anatom cally and m croscop cally w th cultur ng of spec f c and non-specif c bacteria and fung and determ nation of the collagen content n some cases

In an attempt to exclude other diseases as for nstance sarco dos s tuberculosis and var ous systemat c diseases w th lung man fes a ons tests were done in some cases as l s ed n paper III The bacteriolog cal exam na ons ncl de repeated negat ve cultures and gu ea p g tes s for tb from sputum and or gastric la age X ray of skeleton prescalene lymph node b opsy and or med ast nal ex plora on ad modum Carlens 4 and biopsy of the bronch al mucosa and other tissues were also performed

Material

The cases belong to a t tal of 5 500 pat ents w h non tuberculous lung d sease treated in th s hosp al during 1957—1963 Eleven cases ha l the cl n cal d agnos s of IFL during l fet n e n ? cases def n te diagnos s was achieved first at autopsy (cases 8 and 18) on checking all the X ray films from pat ents d agnosed as non tuberculous lung disease during the years 1952—1959 another 6 cases were revealed

The material is shown in table I There were 7 females and 12 males The average age at the onset of d sease was 56 years with range of 27—87 years Th rteen of the pa t ents were 40 to 69 years old at the onset of disease

All the patients were in a good health be fore the present disease and several of them had been athletes None of them had been abroad Other diseases are accounted for in table I there was allergic rhinitis in 2 pa t ents ch ldhood bronch al asthma in one and early rheumatic fever without rema ning symptoms in one Two cases (nos 10 and 11) had been exposed to occupat onal dusts they will be accounted for n paper III (2a)

In 14 of the 19 cases *X ray f lms of the* chest obta ned 1—10 years *before* the present disease were normal even on careful re exam nat on Thus congen tal or in early ch ldhood acqu red d seases could be ex cluded In 2 cases a pos tive chest X ray find ng preceded the present disease by a few years (nos 2 and 5)

Clinical findings (table II)

The most common symptoms were cough dyspnoea and weight loss as described in the literature The weight loss in connection with the initial deteri orat on varied between 6 and 20 kg in 1/2—1 1 2 years

S x patients (nos 2a 5 8 9 10 11) reported at the onset of disease non characteristic chest pains Sometimes it was described as a burning pain behind the sternum and/or discomfort at swal lowing and loss of appetite These symp-toms gradually dim nished or disap-

TABLE I

Case	Sex	Age at onset (yrs)	Occupation	Other diseases	Means of definitive diagnosis: Clin signs, incl X ray	Thoracotomy	Autopsy	Dead	Estim minimal duration (yrs)	Steroid treatment
1	♀	41	Housewife	Whooping cough at 20 Allergic rhinitis	✓		✓	✓	8	
2	♂	27	Salesman	"Pneumonia" 12 yrs before onset	✓				13	✓
3	o	51	Gardener	Prostatitis	×	×			8	✓
4	♀	46	Housewife	Op for ovarian cyst Pernicious anemia	×				9	✓
5	♂	67	Pensioned cotton weaver	—			✓	✓	7	
6	o	73	Merchant	Serum pos syphilis	✓		✓	✓	7	
7	♂	64	Mechanic	—		✓			4	×
8	♀	87	Housewife	Sideropenic anemia			✓	✓	1	
9	♂	57	Merchant	Allergic rhinitis	✓			×	2	✓
10	♂	48	Welder	Prostatitis Cholecystectomy		✓			3	✓
11	♂	65	Road and power plant worker	—		×			3	✓
12	♀	81	Housemaid	Pernicious anemia			×	✓	1	×
13	o	60	Truck driver	Maxillary sinusitis		×	✓	✓	2	✓
14	♂	53	Taxi driver	Chron alcoholism	✓		✓	✓	10	✓
15	♂	43	Warehouseman	—	×				3	
16	♀	51	Clerk	Bronchial asthma at age 7—8	✓				2	
17	♀	53	Leather seam stress	Acute rheumatic fever at 38		✓			1	
18	♂	59	Cobbler	Angina pectoris			✓	✓	12	×
19	♀	49	Housewife	Erythema nodosum? at 25		×			7	
Total					9	7	8	9		11

Methods

The diagnosis of IPL was based mainly on clinical data and radiological findings Other diseases simulating IPL were excluded by means of various clinical and laboratory tests The diagnosis was in most cases confirmed by lung biopsy or was established

first at necropsy See table I and paper III (2 a)

The *clinical history* was obtained from the routine case records For diagnosis IFL was required that the symptoms started at some definite time and that chronic productive or purulent bronchitis had not occurred

The time for *onset of the disease* as indicated by the clinical history was confirmed by the radiological findings This of course, required a check up of all earlier routine films and microfilms

The radiological technique for *radiograms* of the chest was standardized as far as possible in general at 130—140 kv The low kilovolt technique 70—80 kv was used in an attempt to detect early honeycomb pattern but did not prove to be of any advantage Tomograms and bronchograms were obtained in most cases

Diagnostic thoracotomy was performed in most cases using the technique described by Andér and Bergh (2) with inspection and palpation of the lung and biopsy from diseased and non-diseased parts of the lung The excised samples were studied anatomically and microscopically with culturing of specific and non specific bacteria and fungi and determination of the collagen content in some cases

In an attempt to exclude other diseases as for instance sarcoidosis tuberculosis and various systematic diseases with lung manifestations tests were done in some cases as listed in paper III The bacteriological examinations include repeated negative cultures and guinea pig tests for tb from sputum and/or gastric lavage X ray of skeleton prescalene lymph node biopsy and/or mediastinal exploration ad modum Carlens (4) and biopsy of the bronchial mucosa and other tissues were also performed

Material

The cases belong to a total of 5 500 patients with non tuberculous lung disease treated in this hospital during 1957—1963 Eleven cases had the clinical diagnosis of IFL during lifetime in 2 cases definite diagnosis was achieved first at autopsy (cases 8 and 18) on checking all the X ray films from patients diagnosed as non tuberculous lung disease during the years 1952—1959 another 6 cases were revealed

The material is shown in table I There were 7 females and 12 males The average age at the onset of disease was 56 years with range of 27—87 years Thirteen of the patients were 40 to 69 years old at the onset of disease

All the patients were in a good health before the present disease and several of them had been athletes None of them had been abroad Other diseases are accounted for in table I there was allergic rhinitis in 2 patients childhood bronchial asthma in one and early rheumatic fever without remaining symptoms in one Two cases (nos 10 and 11) had been exposed to occupational dusts they will be accounted for in paper III (2a)

In 14 of the 19 cases X ray films of the chest obtained 1—10 years *before* the present disease were normal even on careful reexamination Thus congenital or in early childhood acquired diseases could be excluded In 2 cases a positive chest X ray finding preceded the present disease by a few years (nos 2 and 5)

Clinical findings (table II)

The most common symptoms were cough dyspnoea and weight loss as described in the literature The weight loss in connection with the initial deterioration varied between 6 and 20 kg in 1/2—1 1/2 years

Six patients (nos 2a 5 8 9, 10, 11) reported at the onset of disease non characteristic chest pains Sometimes it was described as a burning pain behind the sternum and/or discomfort at swallowing and loss of *appetite* These symptoms gradually diminished or disap-

TABLE II

Symptoms	No
Unproductive cough	17
Increasing dyspnoea	17
Bloodstained sputum	9
Non characteristic chest pain	3
Discomfort on swallowing	4
Weight loss	9
Non characteristic aching of the extremities	2
Physical findings	
Cyanosis at first observation	7
Clubbing	[illegible]
Basal crepitant rales	13
Rhonchi	1

peared On examination of the oesophagus with X ray or at necropsy there were no local signs of scleroderma

Fine basal crepitant rales were heard in the majority of the cases Only in one patient were there rhonchi spirometry in this patient did not indicate airways obstruction

Onset and clinical course

In 2 cases (nos 3 and 10) there was a relatively *acute onset* with a succeeding progress of the symptoms during 3—6 months they are still alive after 8 and 3 years The remaining 17 cases had a *slow onset* with a gradual increase of the symptoms

In 8 of the slow onset cases there was after 1 1/2 to 11 years course an *exacerbation* as indicated by history and clinical signs and in some cases also by radiological findings In connection with this exacerbation seven patients died in spite of the fact that several of them were on steroid medication In the eighth case, with exacerbation after five years of observation steroid administration was accompanied by improvement

Four cases without steroid medication are still alive They seem to have a slow deterioration as indicated by history and in one case also by ventilatory tests and radiological appearance The average duration of disease as counted from onset of symptoms in those cases who have died is 4 1/2 years 6 patients lived for 1—3 years two for 8—10 years and one for 12 years

The final state was characterized by respiratory failure with severe hypoxia but without signs of severe CO_2 retention (in one case $P_A CO$ was 50 in another 55 mm Hg) In one case there was a sudden death with no explanation at autopsy In 3 cases there was a non specific superinfection In one case a pulmonary embolus was the immediate cause of death (no 13)

Physiological observations

Studies were done on 13 cases at the first observation on acute exacerbation and or before corticosteroid medication was started Detailed account will be given in paper II (13)

The *vital capacity* was below 80 % of the predicted normal in all patients except two There were no spirometric signs of airway obstruction

The *alveolar arterial oxygen pressure difference* $(P_A\ _a O_2)$ at room air was moderately to markedly elevated in all cases except two (nos 16 and 19) During low oxygen breathing pathological values were obtained in 5 of the 6 patients

so studied, in 2 cases the values were markedly elevated The arterial oxygen saturation was decreased roughly in proportion to the elevation of the P_{A-a} O_2 at air breathing Thus there appears, in the majority of these patients *uneven ventilation/perfusion or uneven ventilation/diffusion* as well as *impairment of the diffusion capacity* within the lungs

Arterial carbon dioxide pressure was below 35 mm Hg in 4 cases, indicating alveolar hyperventilation This was certainly due to the low arterial oxygen pressure

In general, there was a good correlation between the clinical course, whether this involved improvement or deterioration and the changes in ventilatory capacity This will be reported in more detail in paper II together with observations on gas exchange during steroid medication (13)

Radiological findings

The radiographic findings at any stage, may be classified as follows

1 A faint haze with a fine granular appearance confined to the lower half of the lungs only (12 16 18) On lateral view or better on lateral tomograms one may observe a bandlike pattern running parallel with the diaphragmatic surface indicating a diminished volume of the lower lobe
2 Lesions localized mainly in the apices Subpleurally there is increased reticulation with more or less obvious ring shadows which may be interpreted as tuberculosis
3 Generalized honeycomb pattern with thin ring shadow walls enclosing zones of translucency 4—10 mm in diameter If the ring shadows are smaller or more numerous, the appearance is indistinguishable from a reticular or netlike pattern' In advanced cases, the appearance is sometimes described as cystic lung although large or coarse honeycomb shadowing would be a better description (18)

Initial findings (table III)

The initial X ray findings were observed on chest films initiated by the onset of symptoms or, in 2 cases, obtained at health controls

In 10 cases there were *basally located parenchymal lesions*, as described above under 1 The lesions were bilateral, except in one case with initial dominance on one side

In case no 10 (described in more detail in paper III (2a) there were for several years stationary discrete disseminated nodular infiltrations interpreted as occupational welder s siderosis In case no 7 the first examination showed non characteristic cloudy infiltration dis eminated over the whole lung field with a few irregular lines The diagnosis was verified by lung biopsy In case no 8 the lesions from the first observation were localized around the hili with signs of honeycombing

In 4 patients there was mainly the *apical localization* as described above under 2 No bacilli were found on a great number of bacteriological tests and no tuberculosis later at autopsy

TABLE II

Symptoms	No
Unproductive cough	17
Increasing dyspnoea	17
Bloodstained sputum	2
Non characteristic chest pain	3
Discomfort on swallowing	4
Weight loss	9
Non characteristic aching of the extremities	2
Physical findings	
Cyanosis at first observation	7
Clubbing	6
Basal crepitant rales	13
Rhonchi	1

peared On examination of the oesophagus, with X-ray or at necropsy, there were *no local signs of scleroderma*

Fine basal crepitant rales were heard in the majority of the cases Only in one patient were there rhonchi, spirometry in this patient did not indicate airways obstruction

Onset and clinical course

In 2 cases (nos 3 and 10) there was a relatively *acute onset* with a succeeding progress of the symptoms during 3—6 months, they are still alive after 8 and 3 years The remaining 17 cases had a *slow onset* with a gradual increase of the symptoms

In 8 of the slow onset cases there was, after 1 1/2 to 11 years course an *exacerbation*, as indicated by history and clinical signs and in some cases also by radiological findings In connection with this exacerbation seven patients died, in spite of the fact that several of them were on steroid medication In the eighth case, with exacerbation after five years of observation, steroid administration was accompanied by improvement

Four cases without steroid medication are still alive They seem to have a slow deterioration, as indicated by history and in one case also by ventilatory tests and radiological appearance The average duration of disease as counted from onset of symptoms in those cases who have died, is 4 1/2 years 6 patients lived for 1—3 years, two for 8—10 years and one for 12 years

The final state was characterized by respiratory failure with severe hypoxia but without signs of severe CO_2 retention (in one case P_aCO_2 was 50, in another 55 mm Hg) In one case there was a *sudden death with no explanation* at autopsy In 3 cases there was a nonspecific superinfection In one case a pulmonary embolus was the immediate cause of death (no 13)

Physiological observations

Studies were done on 13 cases at the first observation, on acute exacerbation and/or before corticosteroid medication was started Detailed accounts will be given in paper II (13)

The *vital capacity* was below 80 % of the predicted normal in all patients except two There were no spirometric signs of airway obstruction

The *alveolar-arterial oxygen pressure difference* ($P_{A-a}O_2$) at room air was moderately to markedly elevated in all cases except two (nos 16 and 19) During low oxygen breathing pathological values were obtained in 5 of the 6 patients

One patient, who initially showed around the hili a reticular pattern with small ring shadows and hairlines later showed an accentuation of this finding The diagnosis of IFL was verified at autopsy

Of the 4 patients, who on first observation showed apical localization, all developed successively honeycomb pattern, with a tendency to concentration in the apical *peripheral* part of the lung The fully developed picture thus consists of a general small cystic honeycomb appearance with varying degrees of a bandlike fibrotic pattern In a couple of cases there were large cysts Furthermore, the hili were elevated bilaterally the upper part of the medi astinum was widened and the dia phragms were elevated with a more or less sharp contour

In one of the 4 cases with bronchographic changes located in the basal parts of the lung only there was after steroid medication a nearly complete normalization of the radiograph and of the bronchograph

In one patient (no 2) with markedly widened pulmonary artery during the hypoxic stage there was normalization of the pulmonary artery after steroid treatment

Course during steroid medication

During the initial two months of treatment with corticosteroids relatively high doses were given — calculated as prednisolone — 20—60 mg Later the dosage was gradually diminished to a maintenance level of 5—15 mg In connection with acute exacerbations on the radiological pictures the dosages was temporarily increased

Eleven patients were treated with corticosteroids Five of them received medication only in a late stage of the disease and died within 6 months after the onset of treatment Of the remaining 6 3 improved and 3 showed successive deterioration they were treated for 2 1/3—8 years

The improvement was evident both from the clinical state and from the X ray picture In case no 2 the medica tion was started in connection with an acute exacerbation 5 years after the onset of symptoms the remission was almost complete and the patient has been maintained on steroid medication for 7 years In case no 4 medication was stopped after 2 years and 4 months and the remission is still maintained clinically and radiologically 2 years after the cessation of medication In the third case (no 10) there was an initial im provement clinically and radiologically After lowering the dosage to mainte nance level there was an acute temporary deterioration on the radiograph but not clinically after increase of the dosage the radiograph again improved

The remaining 3 cases have showed a successive deterioration However I have the impression that in at least one of them the steroid medication has slowed down the very rapid clinical and radiological deterioration in this patient medication is still maintained after 8 years One of the patients stopped his medication himself and this may have accelerated his already at that time progressive deterioration

TABLE III

Case	Initial X ray				X ray course		
	Localization			Occurrence of honey comb	Pro gression	Development of honey comb	Remarks
	Basal	Apical peripheral	General				
1	×				×	×	
2	×						Regression
3	×				×	×	
4	×						Regression
5		×		×	×		
6		×		×	×		
7			×			×	
8				×	×		Central localization
9		×			×	×	
10	×						Regression
11	×					×	
12			×	×			
13			×	×			
14			×	×			
15	×						
16	×						
17	×						
18		×				×	
19	×					×	

Three cases had a *general, advanced honeycomb* pattern bilaterally as described under 3

In 3 patients there was a unilateral or bilateral *pleural thickening*

In 5 cases, in patients in a markedly hypoxic state, the *pulmonary artery* was enlarged

Bronchography was performed in 11 patients In 4 of them there was, in the basal parts of the lung, a decreased vol ume The bronchi were curved and mesh ed but without any bronchiectasis One patient showed obvious ectatic bronchi in the lower lobe with varying width but without a honeycomb pattern

In 5 patients, the bronchography showed marked general engagement of the bronchi *The bronchi were widened* with irregular contours Their general course was straightened towards the periphery, subpleurally the straight course ended abruptly, and instead of the normally unfilled marginal region, there were bronchiolectases and cystic formations

Radiological course (table III)

Of 11 patients who initially showed basally located changes or disseminated non characteristic infiltrations, 5 developed honeycomb pattern

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TABLE IV A Patients without honeycomb pattern at onset of steroid treatment or at hospitalization respectively

Steroid treated					No steroids				
Case	Improved	Worse	Dead	Estim min dur (yrs)	Case	Improved	Worse	Dead	Estim min dur (yrs)
2	×			13	1			×	8
3		×		8	15		×?		3
4	×			9	16		×		2
10	×			3	17		×		1
7		×		4					
11		×		3					

B Patients with local or generalized honeycomb pattern at onset of medication or on hospitalization respectively

Steroid treated			No steroids		
Case	Dead	Estim min dur (yrs)	Case	Dead	Estim min dur (yrs)
9	×	2	5	×	7
12	×	1	6	×	7
13	×	2	8	×	1
14	×	10	19		7
18	×	12			

Weight loss (1—18 kg) *during steroid* medication was observed in 6 patients *Weight gain* (3—8 kg) was observed in 5 patients in one emaciated case the gain was 16 kg, equalling the premedication weight loss The weight gain paralleled the clinical, radiological and functional improvement

All 6 treatment cases are still alive they are working at their regular jobs full or part time

Studies on respiratory function during steroid medication will be presented in paper II (13)

Side effects of steroid medication

In 3 cases blood pressure elevation of more than 20 mm Hg systolic and/or diastolic was observed In one patient diabetes mellitus became evident after 2 years of treatment after initial insulin treatment peroral therapy has proved to be adequate in spite of continuing steroid medication

In 4 cases radiological examination of the thoracic and lumbar regions of the back was performed after more than 2 years of medication In one of these after 7 years of medication there was

a compression of 2 vertebrae and pain referred to this compression

No symptoms of gastritis or duodenal ulcer occurred No mental disturbances were observed In no case did observed complications necessitate cessation of medication

Prognosis

Evaluation with regard to initial X ray finding The untreated and the treated cases have been divided in 2 groups according to radiological appearance at the time of hospital admission or onset of medication respectively One group includes patients without any honeycomb appearance (table IV A) and the other group those with local or general honeycomb pattern (table IV B)

In the group *without honeycomb* appearance there were 10 patients Three improved and 7 deteriorated one of these died The minimal duration of disease in this group varied between 1 and 13 years

The group with local or general honeycomb appearance includes 9 patients Eight of these patients are dead and the remaining one has deteriorated The duration of the disease varied between 1 and 12 years

Evaluation with regard to steroid treatment Of the 10 patients *without honeycomb* pattern 6 were given steroid medication In the treatment group 3 patients improved and 3 deteriorated with development of honeycomb pattern In the untreated group 3 of the 4 patients deteriorated one of these developed honeycomb pattern and died

Of the 9 patients *with honeycomb* pattern 5 received steroid medication Of the treated cases all are dead with a duration of disease of 3—12 years In the untreated group, 3 are dead and one patient is alive but has deteriorated clinically with a duration of disease of 1—7 years

Discussion

The age distribution in the present material agrees in general with other published materials in adults (10, 12, 15, 17) In the present material there are 2 very old patients 81 and 87 years respectively The disease occurs in all ages and one should expect to see it both in children (11) and in very old people

In 4 of the patients there was one symptom not generally stressed in the literature, namely diffuse discomfort and pain on swallowing and unwillingness to eat This occurred in the initial period of the disease and was difficult to separate from general chest pains No signs of progressive systemic sclerosis (scleroderma) were demonstrated either on oesophageal X ray examination or at autopsy

Weight loss was a dominant symptom both in the initial stage and later Steroid medication reversed the weight loss only in those cases where it was accompanied by clinical and radiological improvement Weight loss is apparently an important clinical sign for 5 of the 6 patients with continuing weight loss have died No direct explanation for this weight loss has been found in the literature

In general there were no rhonchi on auscultation and likewise no signs of

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3		×		8	15		×?		3
4	×			9	16		×		2
10	×			3	17		×		1
7		×		4					
11		×		3					

B Patients with local or generalized honeycomb pattern at onset of medication or on hospitalization respectively

Steroid treated			No steroids		
Case	Dead	Estim min dur (yrs)	Case	Dead	Estim min dur (yrs)
9	×	2	5	×	7
12	×	1	6	×	7
13	×	2	8	×	1
14	×	10	19		7
18	×	12			

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ers by bronchography, these patients all had a rapid deterioration One patient showed peripheral subpleural cystic degeneration on the bronchograph before honeycombing could be definitely diagnosed on the regular radiographs in this patient disseminated honeycombing developed progressively, together with a clinical deterioration in spite of steroid medication In one patient with localized cystic degeneration subpleurally, thoracotomy revealed a much more extensive honeycomb dissemination Thus bronchographic findings of subpleural cystic degeneration may prove the disease to be in a malignant stage even before this is revealed by the radiography

Summary

During 1952—1963 19 cases of interstitial lung fibrosis were found among 5 500 patients treated in this hospital for non tuberculous lung disease The diagnosis was based on clinical history radiological examination including tomography and bronchography and thoracotomy including lung biopsy In 8 cases the diagnosis was verified at necropsy

The patients showed the generally described syndrome of fatigue dyspnoea and weight loss Initially 6 patients complained of chest pain and/or discomfort on swallowing, with loss of appetite this symptom generally disappeared gradually

The radiographs were divided into one group without honeycomb appearance on the radiograph or bronchograph and one group with localized or disseminated honeycomb appearance Ten patients did not show any honeycomb appearance when steroid treatment was started or on their first hospital observation One of these patients died and the others are alive Steroid medication could not prevent development of honeycomb pattern in 3 of 6 treated patients in this group Nine patients showed honeycomb pattern on radiograph or bronchograph at the onset of steroid medication or on first hospital observation Eight of these patients are dead

It is concluded that patients without any honeycomb appearance may be in a reversible stage

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bronchial obstruction on spirometry The lack of rhonchi is somewhat surprising with regard to the frequent occurrence of chronic non-productive cough and radiographic appearance of impressive bronchial pathology

Clubbing was observed only rarely, but this may be due to lack of observation in those cases where the diagnosis was revealed only after careful re-examination of all films or at necropsy

Without therapy most of the cases will show a more or less rapid deterioration *If therapy is to be successful,* it should be started before the parenchymal changes have reached an irreversible stage It is, therefore, important to know whether one can recognize the 'stage of irreversibility" on radiological appearance alone, on biopsy findings alone, or on the bases of physiological studies It is my belief that this can *well be done on the basis of radiological* appearance alone, as will be described below The biopsy findings are in this aspect in agreement with the radiological findings and will be described separately (19) The physiological findings are of a more complex nature and will also be discussed separately (13)

It is possible to separate a prognostically favourable group *on the basis of* chest X-ray alone These cases showed in the lower lobe a diminished volume and distorted bronchi without bronchiectasis In one case these changes were reversed during steroid medication (no 4)

Conversely, a *honeycomb* pattern on the chest X-ray indicates a definitely poor *prognosis In particular* when this pattern is disseminated throughout the lung, the prognosis is very poor The patients with disseminated honeycomb pattern already *on the first contact* with the hospital were all dead within 9—12 months in spite of *steroid* medication

The radiological finding of diminution of the volume of the lower lobes does not necessarily mean parenchymal destruction On the other hand, the finding of bronchiectasis in the periphery, with contrast-filling on the bronchograph extending into the subpleural region, indicates advanced parenchymal *destruction*

The anatomical basis for the honeycomb appearance has been studied extensively by Heppelstone (9), using the Gough technique (5) with sagittal sections of the whole lung The large bronchi, leading into the area with honeycomb appearance, may be normal On entering the diseased area, they become distorted and dilated, and communicate with cysts, consisting of terminal and respiratory bronchioles The bronchi may also end blindly in a fibrotic area The loss of the "contrast free subpleural region' corresponds to destruction of 1 2-3 generations of respiratory bronchioles and equally many alveolar ducts, constituting a total *width of 3—5 mm (12, 14) Thus this* radiological finding constitutes a direct measure of the degree of parenchymal loss (17)

The honeycomb appearance may be disseminated over the whole lung, and then shows up on regular radiographs, or may be localized subpleurally and is then best shown on bronchography In 3 patients the honeycomb was disseminated and also was shown in the periph-

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From the University Lung Clinic (Head Gosta Birath M D) Renstromska sjukhuset Göteborg Sweden

Idiopathic Interstitial Fibrosis of the Lungs

II Reversibility of Respiratory Disturbances During Steroid Administration

By

ROLF MALMBERG, ERIK BERGLUND[1], and LARS ANDFR[2]

In a previous paper a group of 19 patients with idiopathic interstitial fibrosis of the lungs was analyzed with regard to prognosis as indicated by radiological findings 1) It was found that the clinical course was much poorer in the patients with localized or generalized "honeycomb" cystic appearance than in those without whether they were treated with steroids or not

The present paper will describe the physiological findings in the patients mentioned above The studies include ventilatory capacity distribution of inspired air and gas exchange at rest and exercise Initial studies i e before steroid treatment or at first hospitalization were obtained in 16 patients Continued studies during steroid treatment were obtained in 8 of the 11 patients treated with steroids

The analysis is directed at

1 the type of physiological disturbance and

2 the reversibility of various functional disturbances

Material

The material is described in detail in a previous publication (1) There were two groups according to radiological findings 10 cases had only a fine granular appearance without cysts and 9 cases had local or generalized honeycomb cystic appearance Six cases in the former group (nos 2 3 4 7 10 11) and 5 in the latter group (nos 9 12 13 14 18) received steroid treatment

Complete initial data were obtained in 13 of the patients (nos 1 2 3 4 7 9 10 13 14 16 17 18 19) Spirometric data only were obtained in another 4 patients (nos 1 6 12 15) Data were obtained during steroid treatment in 8 patients (nos 2 3 4 7 10 11 13 14)

Methods

Ventilatory capacity was evaluated with dynamic spirometry 4) Three to 5 forced expiratory spirograms were obtained and the forced

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TABLE I Values before steroid medication

Case no	Steroid treatm	Honey comb	Vital capacity (% pred)	S_aO_2 Rest (%)	Alv art O_2 press. diff Rest (mmHg)	Exercise	Low O_2	P_aCO_2 Rest (mmHg)	N_2 gradient (%)
2	+		78	81	74	101	—	23	6.0
3	+		76	94	23	45	21 R	40	8.7
4	+		56	92	42	57	29 R	41	8.0
7	+		63	96	24	42	19 R	36	6.5
9	+	+	58	97	6	30	23 Ex	39	3.1
10	+		58	77	80	—	—	25	8.4
11	+		55	—	—	—	—	—	7.3
12	+	+	49	—	—	—	—	—	
13	+	+	53	87	29	54	37 R	46	7.4
14	+	+	56	96	18	39	36 Ex	36	8.8
18	+	+	36	83	—	—	—	32	
1			36	[1]68	—		—	[1]33	
6		+	51	—		—		—	
15			91		—	—	—	—	
16			61	97 Ex	—	2	24 Ex	45 Ex	4.3
17			82	97	17	46	36 Ex	35	3.9
19		+	73	97	9	9	12 Ex	41	3.8

[1] Values obtained a short time before death

20 mm Hg in 7/11 cases studied at rest and 8/10 cases studied at exercise. Values over 40 mm Hg were obtained in 3 cases *at rest and* 6 cases at exercise. With low-oxygen breathing some cases were studied at rest (R) and others during exercise (Ex). Seven of the 9 cases had $P_{A-a}O_2$ over 20 mm Hg. For the same load (rest or exercise) the $P_{A-a}O_2$ was about the same with room air and low-oxygen breathing in most of the cases.

No attempt was made to test the patients' maximal working capacity; the exercise levels were of a low to moderate severity. The breathing frequency was over 30 in 5 of 10 cases studied.

There was marked *alveolar hyperventilation* as shown by very low P_aCO_2 (arterial CO_2 pressure) values in the two most hypoxic cases (nos 2, 10). In case no 1 a value of 33 was obtained in the agonal stage; in case 18, with a superimposed purulent bronchitis, an agonal value of 56 mm Hg was observed.

Single breath nitrogen tests performed initially or later showed in 8 cases pathological N_2-gradients between 6 and 9 %; in 4 cases the values were between 3 and 4.5 %. The other 7 were not studied.

A few *repeated studies* were done on patients without steroid medication. Vital capacity showed a decrease from 61 to 46 % during 14 months in patient no

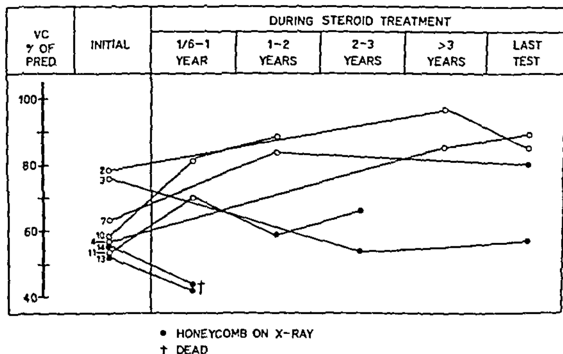


Fig 1 Vital capacity during steroid treatment

expiratory volume in one second ($FEV_{1.0}$) the forced vital capacity (FVC) and the ratio $FEV_{1.0}$ over FVC in % (FEV %) were calculated If vital capacity (VC) recorded during slow breathing on the same spirometer exceeded the FVC the VC value was used for calculating the FEV % All values were related to predicted values from a recently obtained normal material (2) Terminology is used according to Pappenheimer et al (12)

Gas exchange studies were performed at rest and if possible at light to moderate exercise on a bicycle Arterial blood was drawn for analysis of pH carbon dioxide tension (3) and oxygen saturation (8) and expired gas was collected for analysis of oxygen and carbon dioxide concentrations (15) Arterial oxygen pressure was estimated from pH and oxygen saturation using the Dill nomogram From these values alveolo-arterial oxygen pressure differences ($P_{A-a}O_2$) were derived assuming equilibrium between alveolar and arterial carbon dioxide pressures The study was repeated with the patient breathing a low oxygen mixture of around 16 % if this was tolerated

Distribution of inspired oxygen was determined with the single breath nitrogen test modified from Comroe and Fowler (5 9) The nitrogen concentration was recorded in the gas expired after a single breath of oxygen the increase in nitrogen concentration from 750 ml to 1 250 ml expired volume (N_2 gradient) was calculated and related to predicted normal values (14)

Results

Studies before steroid medication

Spirometry (fig 1 and table I) showed a vital capacity lower than 80 % of predicted normal in 15 of the 17 cases so studied Nine cases had values lower than 60 % FEV % on the contrary was normal or in most of the cases even higher than predicted normal value

Oxygen transport with room air breathing was impaired as judged from $P_{A-a}O_2$ (Alveolar arterial oxygen pressure difference) This was elevated above

heart rates for each patient are shown there was a fairly good correlation between changes in $P_{A-a}O_2$ and changes in heart rate

Evaluation of reversibility

With regard to the simple spirometric tests used in this study the treated group may be divided into those with values more than 60 % and those with less than 60 % of predicted Steroid treatment was given to 3 patients in the former group and 2 of these showed improvement of vital capacity none of them had honeycomb appearance before treatment Steroid treatment was also given to 8 patients with vital capacity values below 60 % of predicted Five of them (nos 9 12 13, 14 18) died within one year 2 of these were tested and showed decreased values Three patients with equally low pretreatment values showed marked improvement temporary or long standing The presence of or appearance of honeycomb pattern on X ray was associated with deterioration of ventilatory function

With regard to the data on $P_{A-a}O_2$ with room air breathing it is apparent from table I and fig 2 that extreme pathological values (nos 2 and 10) were found in patients without honeycomb appearance It is also clear that high $P_{A-a}O_2$ values may very well show marked regression during treatment Appearance of honeycomb is probably associated with stationary or increasing $P_{A-a}O_2$ values

With regard to $P_{A-a}O_2$ with low-oxygen breathing repeated values were obtained in 7 cases In 6 of these the

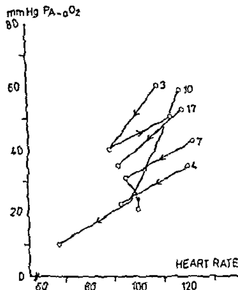


Fig 3 Alveolo-arterial oxygen tension difference and heart rate at identical work loads Values for each individual patient connected by a line

values changed but a few mm Hg and only in no 10 was there a decrease from 44 to 25 mm Hg

Discussion

Before treatment the patients showed more or less impairment of ventilatory capacity This limitation was apparently not due to airways obstruction for a large fraction of the vital capacity could be expired in one second The findings are in agreement with previously published reports (6 7)

The finding of uneven distribution of inspired gas as shown by the single breath nitrogen test without any demonstrable generalized (?) airways obstruction is remarkable Similar results were obtained by Herbert et al who on the other hand recorded normal wash-out

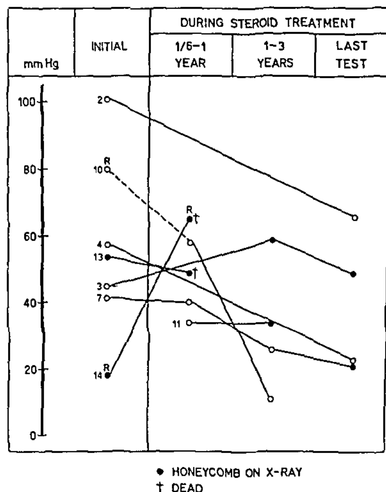


Fig 2 Alveolo arterial oxygen tension difference during steroid treatment

16, and from 82 to 72 % during 3 months in patient no 17

Studies during steroid administration

Eight patients were followed for 4 months to 8 years As shown in fig 1, *vital capacity* improved to normal values in 4 cases (nos 2, 4, 7, 10), and fell in 3 cases (3, 13, 14) Case no 11 showed improvement during the first year later followed by a decrease FEV % showed only minor variations during the course with one exception (no 14), who fell from 86 to 66 %

Oxygen transport, as judged from $P_{A-a}O$ with room air breathing, is shown in fig 2 Of the 6 cases with values initially over 40 mm Hg, 4 cases improved markedly (nos 2, 4, 7, 10), and the other 2 did not change Case 14 with $P_{A-a}O$ of 18 mm Hg deteriorated $P_{A-a}O_2$ at low oxygen breathing did not change in 4 cases (nos 3, 7, 11, 13), and improved in case no 10 from 44 to 25 mm Hg In case no 4 it changed from 29 at rest to 25 at exercise

In cases nos 2 and 10, the improvement in arterial blood oxygenation was accompanied by partial normalization of the P_aCO_2 values at rest from 23 to 32 and from 25 to 35, respectively

In 5 patients gas exchange studies were performed repeatedly at identical work loads In fig 3 the $P_{A-a}O$ and

heart rates for each patient are shown, there was a fairly good correlation between changes in $P_{A-a}O_2$ and changes in heart rate

Evaluation of reversibility

With regard to the simple spirometric tests used in this study, the treated group may be divided into those with values more than 60 % and those with less than 60 % of predicted Steroid treatment was given to 3 patients in the former group and 2 of these showed improvement of vital capacity none of them had honeycomb appearance before treatment Steroid treatment was also given to 8 patients with vital capacity values below 60 % of predicted Five of them (nos 9 12 13, 14 18) died within one year 2 of these were tested and showed decreased values *Three patients* with equally low pre treatment values showed marked improvement temporary or long standing The presence of or appearance of honeycomb pattern on X ray was associated with deterioration of ventilatory function

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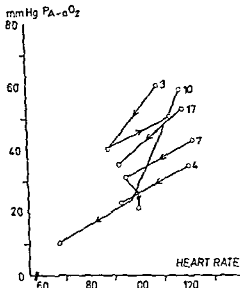


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The finding of uneven distribution of inspired gas as shown by the single breath nitrogen test without any demonstrable generalized (I) airways obstruction is remarkable Similar results were obtained by Herbert et al who on the other hand recorded normal wash out

values The latter data indicate that alveolar ventilation is uniformly distributed The pathologic single breath test may thus depend on regional disturbances in oxygen uptake (16) or possibly on regional compliance disturbances with uneven time constants (11)

Elevation of the alveolo-arterial oxygen pressure difference may be relatively increased due to impaired "diffusion" or to alveolar hypoventilation in areas with capillary blood flow It is likely that "diffusion" impairment shows up mainly with low-oxygen breathing and that ventilation/blood flow disturbances are most evident with room-air breathing (13) The $P_{A-a}O_2$ values were in several cases (nos 3, 4, 9, and 17) higher with room air than with low oxygen breathing, which indicates that unfavourable ventilation/blood flow ratios constituted the main gas exchange disturbance This interpretation is in agreement with that of *Holland* and *Blacket* (7) and *Luchsinger* et al (10) Herbert et al, on the other hand, believe that "diffusion impairment was perfectly adequate to explain practically all of the abnormal A a gradient" (6)

The reversibility of ventilatory impairment during long-term steroid administration was related to the absence or presence of honeycomb lesions rather than to the degree of functional impairment In the treatment group of Herbert et al (6) the pulmonary function in general did not improve, this difference may be due to a shorter observation time as well as selection of material

It is interesting to note that extreme $P_{A-a}O_2$ values could appear in patients without honeycomb lungs, and that these extreme values decreased markedly during steroid treatment This decrease supports the hypothesis of unfavourable ventilation/blood flow ratios as the main cause Alveolar hyperventilation with low arterial CO_2 pressures was found only in connection with severe arterial hypoxaemia, and the disturbances showed parallel regressions during treatment

There was, in each patient, a fairly good agreement between changes in $P_{A-a}O_2$ values and heart rate during exercise, with breathing of room-air Thus it is possible for practical purposes to obtain a rough estimate of variations in gas exchange during treatment by using the simple bicycle ergometry test instead of more complex blood gas studies

In a previous paper (1) it was found that the prognosis, with or without treatment, was poor when honeycomb lesions appeared radiologically The present data, from the same patients, indicate that physiological data do not add to the evaluation of prognosis Patients with fair and poor ventilatory function may improve or deteriorate during treatment Patients with moderately elevated alveolo alveolar oxygen pressure gradients may likewise improve or deteriorate during treatment

Summary

Respiratory disturbances were evaluated with dynamic spirometry, the single-breath nitrogen distribution test and gas exchange studies at rest and exercise in 17 out of 19 patients described in a previous paper

Before treatment with steroids moderate or marked ventilatory impairment without airways obstruction, was found in 15 of the 17 cases studied. Pathological single breath nitrogen tests were observed in 8 out of 12 cases studied. The oxygen transport studies showed moderately or markedly elevated alveolo-arterial oxygen pressure differences and in 4 cases markedly lowerered oxygen saturation. It is believed that both uneven ventilation blood flow and diffusion impairment contribute to this. Alveolar hyperventilation was found in 2 markedly hypoxaemic cases.

During steroid treatment improvement of ventilatory capacity and gas exchange was found only in cases without honeycomb lesions. Two cases with extremely great pre treatment $P_{A-a}O_2$ values showed marked reduction indicating extensive improvement of ventilation blood flow conditions.

On repeated identical exercise tests there was in each patient a fairly good correlation between changes in heart rate response and change in $P_{A-a}O_2$ values on breathing of room air.

Acknowledgement

Supported by grants from Carin Trygger's Memory Fund and the Swedish Medical Research Council.

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From the Department of Medicine Karolinska Institutet at Serafimerlasarettet, Stockholm, Sweden

On the Linolenic Acid Intake in Sweden 1945—1963

by

Gunnar Biorck and Bo Edgren

Recently it has been reported (3, 4, 5, 6, 7, 8) that linolenic acid more than any other polyunsaturated fatty acid can diminish an abnormally high platelet adhesiveness (anti Willebrand factor) in man both *in vitro* and *in vivo* Owren (7) has also published data indicating that the linolenic acid intake among Norwegians has tended to decrease over the years whereas the cardiovascular mortality has risen sharply since World War II during which cardiovascular mortality was significantly low Owren (5 7) has suggested that coronary thrombosis may be related to a deficiency of linolenic acid

Eeg Larsen (2) has stated that the amount of linolenic acid per 3 300 cal among Norwegian labourers has decreased from 1 58 g in 1927/28 to 1 11 in 1947 48 and 1 06 in 1958 Eeg Larsen however is more cautious in drawing any conclusions as to the prophylactic effect of linolenic acid against atherosclerotic heart disease his main argument against Owren s theory being that during 1942—45 when linolenic acid intake reached an all time low value of 0 88 g per 3,300 cal, the cardiovascular mortality was also very low

A comparative study on the mortality in cardiovascular diseases from Scandinavian countries, which was recently performed in our department (1) has shown that the trend during the years 1952—1960 has been different in Norway in comparison with Denmark Finland and Sweden (figs 1—3) Among possible reasons for this the alleged deficiency of linolenic acid in the Norwegian diet might play a role (5) even if definite evidence for this is lacking

We have therefore considered it to be of interest to calculate the average intake of linolenic intake among Swedes from 1945 to the present date The values are given in fig 4 and represent the intake via margarine butter cheese milk cream and cooking fats and should represent at least 95 % of the total intake of linolenic acid Not included are cereals and horse fat both of which contribute very little to the linolenic acid intake

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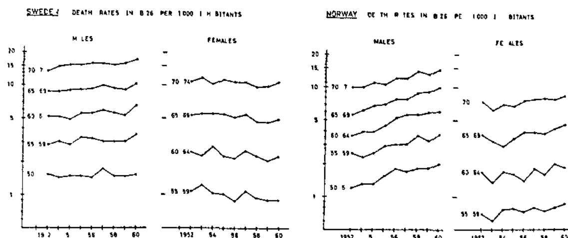


Fig 1 Death rates in cardiovascular heart disease in Sweden and Norway 1952—1960 B 26 equals group A 81 in the WHO classification and includes atherosclerotic and degenerative heart disease

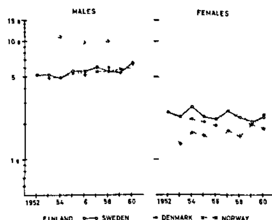


Fig 2 Death rates in cardiovascular heart disease in Finland Sweden Denmark and Norway 1952—1960 in the age group 60—64 years

quantitatively The values have been calculated from the official statistics of the consumption of fats and oils published by the Swedish Department of Agriculture and the Central Bureau of Statistics The data for 1960 have been calculated in another investigation at the National Institute of Public Health from the ready made products consumed and we compared this with our data for 1960, calculated from the raw materials, the value for daily intake of linolenic acid turned out to be the same, 1 5 g (9) in both the investigations

From fig 4 it is seen that between 1945 and 1950 the linolenic acid intake was less than one g per day, but since then the intake of linolenic acid has increased to well over one g During the sixties the intake has exceeded 1 4 g per day and individual It thus seems as if the Norwegians tend to decrease their linolenic acid intake whereas our intake is kept at a higher level than in Norway The main source of the acid in Sweden is the margarine, which has varied considerably up to the sixties This explains the extensive variation in linolenic acid intake over some periods as seen in fig 4

There did not seem to be any relationship between the cardiovascular mortality in Sweden in the years 1945—1960 and the variations in linolenic acid intake

Summary

Because of recent interest in the relationship between linolenic acid deficiency

Fig 3 Death rates n card ovascular disease in Sweden 1930 1960 versus da ly caloric intake cal c ntake as fat an l annual tobacco consumption

and atherosclerotic heart disease We have calculated the Swedish consump tion of linolenic acid during the period 1945 1963 Swedish intake is higher than the Norwegian No immediate relationship between cardiovascular mortality and linolenic acid intake has been traced

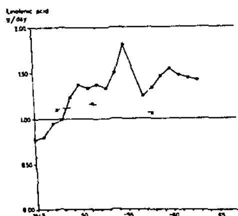


Fig 4 The l nolenic ac d intake per nd v dual and day in Sweden 1945—1963 w l d l ne com pared w th the same intake per 3 300 cal among Norweg an labourers broken l ne from data g ven by Eeg Larsen (2

Acknowledgement

Prof A Wretl nd at the National Inst tute of Publ Heal h has k ndly compared our data for 1960 w h h s in est ga ion f r the same year and has also c t r bu ed to t s invest gation w th valuabl e a l ce

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From King Gustaf V Research Institute (Head G Birke, M D) and the Department of Internal Medicine Karolinska Hospital (Head H Lagerlöf M D) Stockholm Sweden

Inhibition of the Mobilization of Free Fatty Acids from Adipose Tissue in Diabetes

II Effect of Nicotinic Acid and Acetylsalicylate on Blood Glucose in Human Diabetics

By

LARS A CARLSON and JAN ÖSTMAN

It has been suggested by Randle et al (22 23) that the increased concentration of FFA in plasma in diabetes mellitus might to some extent be responsible for the abnormal carbohydrate metabolism In part this theory was based on the findings that increasing concentrations of FFA have been shown to inhibit the uptake of glucose in perfused hearts (10 18 25) and in isolated diaphragm (10) of rats Moreover, it has been observed in human diabetic subjects that the concentrations of FFA in fasting state may be elevated in spite of normal or high concentrations of immunologically assayed insulin and blood glucose (13)

To test this theory it seemed pertinent to study if an acute reduction of the concentration of FFA in plasma would lower the concentration of blood glucose in patients with diabetes For that reason the concentration of blood glucose in diabetic subjects was studied after the administration of either nicotinic acid (NiAc) or acetylsalicylate (AcSal) two agents which inhibit the mobilization of FFA from adipose tissue (2, 3, 4, 5) A preliminary report (6) has appeared on these results In a second series of experiments the effect of NiAc on the glucose tolerance after an intravenous glucose load was studied in diabetic patients

Submitted for publication January 19 19[illegible]

Material and methods

Clinical material

First series Twelve patients in hospital 10 males and 2 females with new-discovered and untreated diabetes mellitus were included None of the patients was in a keto-acidotic state Seven patients were later treated with insulin and five with sulphonyl urea

Second series Eight male patients were included Seven patients had a diabetes mellitus which was insulin-dependent but

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TABLE I. Titratable acidity in samples of plasma and 0.9% NaCl after addition of calcium acetyl salicylate. Mean given of duplicate determinations

Calcium acetyl salicylate added	Dole method		Modified procedure[1]	
(mg/ml)	Plasma (μEq/ml)	NaCl (μEq/ml)	Plasma (μEq/ml)	NaCl (μEq/ml)
0	1.13	0.02	0.81	0.03
$5 \cdot 10^{-4}$	1.15	0.05	0.81	0.01
10^{-3}	1.17	0.07	0.79	0.01
$2 \cdot 10^{-3}$	1.21	0.08	0.81	0
$5 \cdot 10^{-3}$	1.29	0.09	0.80	0

[1] Heptane phase obtained by the Dole method was washed twice with 0.05%, sulphuric acid

Results

Methodological experiments

Table I shows that AcSal interferes with the determination of FFA by the Dole method. After two washings of the heptane phase with the dilute sulphuric acid the content of AcSal was completely removed. Separate experiments with sodium salicylate gave similar results. One washing with 0.05 per cent sulphuric acid was not sufficient to get rid of the interferences of either AcSal or sodium salicylate.

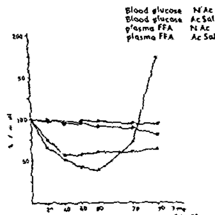


Fig. 1. Mean arterial blood glucose and plasma FFA concentrations in % of initial value after administration of nicotinic acid and acetylsalicylate in human diabetic individuals. (Cf. table IV)

First series of human studies

Table II and fig. 1 show that a rapid fall in plasma FFA was produced by AcSal as well as by NiAc. The concentration of plasma FFA was lower and the mean decrease greater after NiAc than after AcSal from 20 to 80 minutes; the difference was significant at 80 minutes ($p < 0.025$).

The concentration of blood glucose which, like plasma FFA, *did not differ between the groups of patients initially* decreased slowly in the group receiving NiAc (tables III—IV, fig. 1). The decrease was significant at 120 minutes (16.5 ± 5.6 mg/100 ml, mean ± S.E. of mean) and at 150 minutes (14.7 ± 4.2 mg/100 ml). The fall in blood glucose was more apparent after the administration of AcSal and the decrease in blood glucose was significant from

only moderately prone to develop ketosis. In 6 of these subjects the duration of manifest diabetes ranged from 8 to 10 years and all these were treated with one dose of middle-long acting insulin (Lente Novo or N.P.H.) given in the morning. Two patients had newly discovered diabetes and were treated by middle long acting insulin and sulphonyl urea, respectively, for one week before the investigation. Four of the patients received NiAc at the first glucose tolerance test, and the remaining 4 were given the agent at the second investigation. In 6 patients the last injection of insulin was given in the morning on the day before the investigation. In 2 patients the last dose of insulin or sulphonyl urea respectively was given around 48 hours before the studies. The subjects were instructed not to alter their dietary habits before the first investigation or between the first and second trial.

Experimental conditions

All subjects were investigated in supine position in the morning after fasting over night. Indwelling teflone catheters were placed in one brachial artery and in one vein of the opposite arm when injections of NiAc or glucose would be given. After a quiet period of 10 minutes arterial blood samples of 10 ml each were collected into heparinized syringes at regular intervals as described in the following.

First series. Six patients were given calcium acetylsalicylate (Bamyl Hassle) orally in a dose corresponding to 5 g of acetylsalicylate. Six other subjects were given NiAc intravenously in three doses of 200 mg each 30 minutes apart. *Blood samples were removed* 10, 5 and 2 minutes before the administration, the mean of these values was used as the initial value and at 20 or 30 minutes of interval during the following 2 1/2 hours.

Second series. Blood samples were removed 10, 20, 30 and 60 minutes before the glucose infusion and at 10 minute intervals between 20 and 70 minutes after the start of the infusion. Further samples were removed 90 and 120 minutes after the infusion of glucose. One hundred ml of 25 % (w/v) glucose solution was injected intravenously over a period of 8 minutes and at fairly constant rate. Nicotinic acid (Nicangin Draco) was given orally four times in a dosage of 0.5 g each 30 minutes apart, with start immediately after the first blood sampling. Moderate flushing was observed in all patients given NiAc. In addition 2 of the patients given NiAc orally had nausea and vomited some hours after the end of the investigation.

Analytical methods

Blood glucose determination. Glucose was determined in whole blood with glucose oxidase method (17). Duplicate analyses were made on each of three blood samples taken off.

Plasma FFA determination. In separate experiments the interference by acetyl salicylate and sodium salicylate was studied in two current procedures for determination of FFA, i.e. the original Dole method (9), and the procedure modified by Trout (28), since we had previously noted interference (5). In testing the methods the agents were added at various concentrations to plasma and 0.9 % NaCl. From the results obtained a method was devised for determination of FFA in plasma (and tissue) in the presence of salicylates. This modified Dole procedure implies removal of the salicylates by two successive washings of the heptane phase with 0.05 % sulphuric acid. The procedure of Dole as modified by Trout was used in the studies of NiAc since NiAc does not interfere with this method (3).

Calculations

In the glucose tolerance tests the decline of blood glucose plotted against time in a semilogarithmic diagram was essentially linear between 30 and 70 minutes after the injection of glucose. The line connecting the five points from 30 to 70 minutes was drawn. The slope of this line was taken as the fractional turnover rate k as suggested from previous detailed studies (16). No correction was made for glucose lost in urine during the test.

Statistical calculations were made as recommended by Snedecor (26).

of human diabetics

40	60	80	120	150
0 3f ±0 10 −0 31±0 04	0 29±0 08 −0 38±0 04	0 27±0 07 −0 40±0 05	0 49±0 15 −0 18±0 14	1 24±0 38 +0 57±0 31
0 001	<0 001	<0 001	>0 05	>0 05
0 38±0 06 0 26±0 04	0 37±0 05 −0 27±0 02	0 39±0 08 −0 23±0 02	0 40±0 09 −0 25±0 06	0 40±0 09 −0 21±0 09
0 005	<0 001	<0 001	<0 01	>0 05
0 05	>0 05	<0 025	>0 05	>0 05

(mg/100 ml) of human diabetics

40	60	80	120	150
242 5 46 9	241 1±55 6	239 3±45 1	232 0±43 9	233 8±44 7
6 0 6 3	−7 4± 3 9	−9 2± 7 5	−16 5± 5 6	−14 7± 4 2
0 05	>0 05	>0 05	<0 05	<0 025
23f 0 11	230 3± 9 6	220 7± 6 8	214 0±15 1	195 3± 7 8
7 5 4 5	−13 2± 3 8	−22 8± 4 6	−28 0± 2 5	−48 2±11 6
0 05	0 025	<0 005	<0 001	<0 01
0 05	>0 05	>0 05	>0 05	<0 05

shows also the rate of glucose disappearance k) and the mean concentration of plasma FFA from the time when glucose was given and to the time when blood glucose fall had stopped in all subjects 150 minutes after the start of the investigation In 5 subjects with similar basal blood glucose level at the two experiments, the rate of glucose disappearance was equal whether or not the FFA concentration was depressed by NiAc In 2 subjects (cases 5 and 6) there was a significantly increased rate of glucose disappearance during the NiAc experiment The plasma FFA of these subjects was higher than in the other subjects during the study when no NiAc was administered In case 1 who had strikingly higher basal blood glucose concentration at the experiments when

TABLE II. Effects of nicotinic acid and acetylsalicylate on arterial plasma FFA concentration (mEq/l)

Agent administered		Min after administration 0	20
NiAc n=6	Mean ± S.E.M.	0.67 ± 0.11	0.46 ± 0.10
	Mean change ± S.E.M. from initial value	—	−0.21 ± 0.04
	p	—	< 0.005
AcSal n=6	Mean ± S.E.M.	0.64 ± 0.06	0.50 ± 0.08
	Mean change ± S.E.M. from initial value	—	−0.14 ± 0.06
	p	—	< 0.05
Significance of difference between changes observed after NiAc and AcSal		—	> 0.05

TABLE III. Effects of nicotinic acid and acetylsalicylate on arterial blood glucose concentration

Agent administered		Min after administration 0	20
NiAc n=6	Mean ± S.E.M.	248.5 ± 47.6	244.7 ± 44.9
	Mean change ± S.E.M. from initial value	—	− 3.8 ± 3.8
	p	—	> 0.05
AcSal n=6	Mean ± S.E.M.	243.5 ± 10.5	242.5 ± 12.0
	Mean change ± S.E.M. from initial value	—	− 1.0 ± 3.1
	p	—	> 0.05
Significance of difference between changes observed after NiAc and AcSal		—	> 0.05

60 minutes onwards. The maximal decrease was noted at 150 minutes (48.2 ± 11.6 mg/100 ml), when the effect of AcSal was significantly greater than that of NiAc ($p < 0.05$).

Second series of human studies

Table V shows the arterial plasma FFA levels, before and after the intravenous glucose load, and during or without oral NiAc administration. In four subjects (cases 1, 2, 3 and 8) the plasma FFA fell significantly after the glucose administration in experiments, when no NiAc was given. NiAc decreased the plasma FFA in all subjects to a level around 0.20 mEq/l from 80 to 180 minutes after the first dose. Table V

NiAc was given, had at that time also a more delayed rate of glucose disappearance.

Discussion

A rapid fall of plasma FFA was demonstrated after NiAc as well as after AcSal administration thus in conformity to previous and similar studies of each agent in man (4, 5). However, Gilgore *et al.* (12) have reported that infusion of salicylate for one hour *increased* the concentration of plasma FFA in non-diabetic as well as diabetic subjects two hours after the termination of the infusion. It cannot be elucidated whether or to what extent the difference in their and our experimental conditions would account for the disparity of the results. However, it should be pointed out that Gilgore *et al.* used the Dole technique for determination of FFA. Thus they may have had interference from salicylate in their titration values as we (5) and others (2) have observed.

The decrease in blood glucose was significantly greater after the administration of AcSal than after NiAc. Thus the results agree with several previous reports on acute experiments in human diabetics showing that salicylates have a definite blood glucose lowering effect ([illegible], 8, 11, 15) and that NiAc has a small effect (14, 21, 29) which in one study could not be distinguished from that of placebo (14). Whereas the decrease in the concentration of blood glucose was significantly greater in the AcSal experiments, the fall in plasma FFA was more marked after the administration of NiAc. These findings indicate that the effects on blood glucose were not directly related to the reduction of the plasma FFA level. It has also been suggested that salicylates directly stimulate the uptake of glucose into peripheral tissues via extrapancreatic mechanisms (18, 24, 27). The present results also show that a decrease in the plasma FFA level *per se* does not produce any consistent increase in the rate of glucose disappearance. Thus, the results agree with recent studies on the effect of NiAc treatment in alloxan diabetic rats (7). In these rats NiAc caused a marked decrease in the concentrations of plasma FFA and triglycerides, while only minor decrease in the concentration of blood glucose occurred. It is of interest that in the two subjects of the present study with the highest plasma FFA concentration after the injection of glucose when no NiAc was given, there was an increased rate of glucose disappearance at the time when NiAc was administered. This suggests that the higher the FFA levels are the more likely will a reduction of this level cause an improvement of the glucose tolerance. Similar conclusions may be drawn from other studies (19). Their studies show that the rate of glucose disappearance after intravenous glucose load was decreased in normal subjects by norepinephrine infusion, whereas a normal rate of glucose disappearance was demonstrated if nicotinic acid was administered simultaneously. The glucose tolerance of non-diabetic subjects was uninfluenced by nicotinic acid *per se*. When the data from that and the present study are taken together some support

TABLE IV Average changes in arterial blood glucose and plasma FFA concentrations after administration of nicotinic acid and acetylsalicylate in human diabetic individuals Mean individual change ±S E M in % of initial value Cf fig 1

Agent administered	Min after administration					
	20	40	60	80	120	150
Blood glucose						
NiAc	− 1.0±1.4	− 2.2±3.0	− 2.6±1.9	− 2.8±3.3	− 6.3± 1.9	− 6.3± 1.5
AcSal	− 0.4±1.2	− 3.1±1.8	− 5.3±1.5	− 9.1±1.5	−11.9± 1.5	−19.3± 4.5
p	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.01
Plasma FFA						
NiAc	−32.3±6.4	−47.7±5.5	−58.8±5.1	−61.2±4.6	−28.3±21.6	+73.3±47.7
AcSal	−24.4±9.0	−42.0±5.8	−43.5±[illegible].8	−39.9±6.5	−40.0± 8.9	−56.4±13.8
p	> 0.05	> 0.05	> 0.05	< 0.025	> 0.05	[illegible] 0.05

TABLE V Effect of nicotinic acid on the rate of removal of intravenously administered glucose and on the concentration of free fatty acids in plasma of human diabetics Nicotinic acid (NiAc) was administered in a dose of 0.5 g each at 0, 30, 60 and 90 min Glucose (25 g/100 ml) solution was administered at 60 min over a period of 8 min

Case no	Agent administered	Plasma FFA (mEq/l) Min after NiAc administration						Mean plasma FFA (60-150min) (mEq/l)	Blood glucose at 60 min (mg/100 ml)	Glucose disappearance k (% /min)
		0	60	80	100	120	150			
1	0	—	0.68	0.68	0.[illegible]9	0.56	0.68	0.64	299	0.62
	NiAc	0.71	0.51	0.32	0.[illegible]0	0.25	0.22	0.32	502	0.38
2	0	0.48	0.54	0.40	0.60	0.73	0.89	0.63	93	0.77
	NiAc	0.77	0.27	0.26	0.26	0.24	0.21	0.25	160	0.77
3	0	0.64	0.83	0.97	0.61	0.51	0.70	0.72	1[illegible]7	0.76
	NiAc	—	0.20	0.11	0.11	0.09	0.04	0.11	131	0.75
4	0	0.42	0.54	0.55	0.69	0.85	0.77	0.68	247	0.77
	NiAc	0.67	0.33	0.13	0.22	0.18	0.18	0.21	246	0.80
5	0	0.75	0.81	0.82	1.06	0.94	0.94	0.91	195	0.31
	NiAc	0.48	0.22	0.10	0.14	0.15	0.09	0.14	245	0.49
6	0	0.84	0.74	0.73	0.73	0.94	1.00	0.83	195	0.35
	NiAc	0.74	0.30	0.21	0.20	0.20	0.20	0.22	161	0.92
7	0	0.30	0.33	0.38	0.35	0.29	0.25	0.32	179	0.62
	NiAc	0.60	0.20	0.21	0.21	0.19	0.15	0.19	131	0.69
8	0	0.48	0.77	0.49	0.50	0.50	0.66	0.58	145	0.77
	NiAc	0.54	0.24	0.17	0.14	0.09	0.03	0.14	245	0.65

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is given for the concept that pronounced elevations of plasma FFA would in part be responsible for an impaired glucose tolerance

Summary

The effects on arterial concentrations of blood glucose and plasma FFA in human diabetic subjects were compared after the administration of nicotinic acid and acetylsalicylate, respectively Both agents produced a marked decrease in the concentration of plasma FFA, more apparent after the administration of nicotinic acid A slight decrease in the concentration of blood glucose was observed after nicotinic acid administration, whereas the blood-glucose lowering effect of acetylsalicylate was more pronounced

No consistent change in the fractional turnover rate of injected glucose appeared after nicotinic acid administration In two subjects which had higher concentrations of FFA in plasma than the six other diabetics, the decrease in plasma FFA was, however, concomitant with an improved glucose tolerance

The results suggest that in general a decrease in the concentration of plasma FFA in human diabetics does not *per se* increase the glucose uptake into peripheral tissues

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Blood and Tissue Changes in the Dog during and after Excessive Free Fatty Acid Mobilization

A Biochemical and Morphological Study[1]

By

LARS A CARLSON STEN OTTO LILJEDAHL and CLAES WIRSEN

Intracellular accumulation of lipid in otherwise normal cells especially in the liver (fatty liver), is a common finding in states characterized by enhanced mobilization of endogenous lipids from the stores in adipose tissue The role of sympathetic amines in this lipid mobilization at least in some species, has long been recognized (28) Feigelson et al (20) showed that infusion of noradrenaline (NA) to dogs caused a marked increase in the liver triglyceride (TG) content in connection with raised levels of plasma free fatty acids (FFA) which are considered to be the transport form of lipid from adipose tissue to the various tissues in the body (22) In a preliminary report on histochemical tissue changes in the noradrenaline infused dog (63) it was noted that the distribution of intracellular lipid in various tissues was heterogeneous and seemed to be related to certain histochemical parameters Moreover as judged from supplementary electron microscopical findings (42, 63) the affected cells were not visibly damaged even after prolonged infusion but overloaded with fat

These preliminary histochemical findings together with the observation that similar changes occur *after trauma* (8) prompted an extended investigation of the events following administration of NA With a combined biochemical and morphological approach it was considered possible to further elucidate the correlation between blood and tissue changes and also between plasma concentration histochemical characteristics of cells and cellular uptake of fatty acids It was also considered of interest to study *whether and how accumulated lipid is* eliminated and what clinical importance may be attributed to excessive FFA mobilization and or its sequelae

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[1] A preliminary report has been presented elsewhere 11

con rol dogs and in d gs rece ving N A for 8 hours Mean value ± S E of mean and number of animals on t values calculated for the individual *changes*

4	5	6	7	8
0 52±0 09 n=11 0 05		0 57±0 08 n=10 <0 05		0 77±0 08 n=11 <0 05
3 17±0 22 n 22 0 001	3 29±0 27 n=23 0 001	3 16±0 22 n 23 <0 001	2 98±0 20 n=23 <0 001	2 47±0 17 n 23 <0 001
0 22±0 16 n 4 0 05		0 22±0 09 n 4 >0 05		0 20±0 07 n 5 >0 05
0 40±0 07 n 9 0 001	0 58±0 06 n=9 <0 001	0 59±0 07 n 10 <0 001	0 61±0 08 n=9 <0 001	0 58±0 08 n 10 <0 001
75±6 n 19 0 05	77±7 n=20 0 05	76±8 n=21 >0 05	79±7 n=21 >0 05	75±7 n 21 >0 05

tory frequency and pulse rate were made Blood pressure was recorded by means of an Flema Sch onander pressure transducer from a tej l lon cath eter inserted into the femoral artery Th e transducer was f lled with sal ne Rectal ten perature was measured with an ord nary mercury rectal th ermometer All these measurements were performed in con r ect on with blood samp ling

Biochem cal methods

Plasn a FFA were titrated accord ng to Dole 14 plasn a glycerol was determ ned en zym at cally accord ng to W elan l (62) and blood glucose was est mated by the glucose ox dase r ethod 41 Lip ds were determ ned essent ally as lescribed p rev ously (6) w th the Lich gaeff c lor react on for ch lesterol 25 anal sis of inorgan c p l osp hate for pl os- p hol pi ls and est n ation of th e TG-glycerol for TC as mod f ed Total p lasma p rotein was deter nined by the bi ret reaction The plasma l p p rotein s were separated in th e preparat e ultracen trifuge accord ng to th e principles of Bragdon Havel and Boyle (4) and analyzed as described previously (10) The l poproteins harvested in the top of the tube after the first centrifugation (density 1 006) are called very low density (VLD) and those harvested in the top of the tube after the second centrifugation (density 1 063) low density (LD) l poproteins The remaining l pids are called h gh density (HD) lipoproteins The liver lipids were extracted by homogenizing p eces of liver in methanol in an all glass homogenizer and extracting with chloroform meth anol (7)

Calculations

The statistical analyses were done as recommended by Snedecor (57) All plasma lipid values have been corrected for changes in th e concentration of p lasma proteins

Histological methods

For general survey and estimation of intra cellular deposit on of l pids, pie es from th e biopsy specimens were fixed in ice-cold neu

TABLE I Concentration of FFA and glycerol in plasma and of glucose in blood at different times in
• analyzed p indicates the statistical significance of the changes from 0 hours and is based

		Time (hours)			
		0	1	2	3
FFA (mEq/l)	Controls	0 46±0 08 n=11	0 45±0 08 n=11	0 47±0 11 n=9	
	p		>0 05	>0 05	
	NA	0 57±0 04 n=23	2 77±0 27 n=23	2 80±0 17 n=23	2 99±0 17 n=23
	p		<0 001	<0 001	<0 001
Glycerol (mM/l)	Controls	0 07±0 01 n=4	0 07±0 01 n=4	0 08±0 01 n=4	
			>0 05	>0 05	
	NA	0 12±0 03 n=10	0 50±0 07 n=10	0 56±0 05 n=10	0 61±0 06 n=10
	p		<0 001	<0 001	<0 001
Glucose (mg/100 ml)	NA	84±6 n=20	103±12 n=21	84±7 n=21	72±6 n=19
	p		<0 05	>0 05	<0 05

Material and methods

Thirty five healthy mongrel dogs of both sexes, weighing around 20 kg were used after fasting overnight They were anesthetized with Nembutal® 30 mg/kg body weight in travenously, and superficial anesthesia was maintained throughout the experiments All animals were intubated A solution of 1 noradrenaline base (Norexadrin®, Astra Sodertalje, Sweden) was made up in saline at a concentration of 1 mg/kg body weight and 1,000 ml It was given through a catheter in the femoral vein at an infusion rate of 0 5 (4 dogs) or 1 0 µg/kg body weight and minute We did not see any significant difference in the FFA levels obtained at these dosage levels The animals were divided into five groups NA infusion 8 hours (6 dogs), NA infusion 24 hours (6 dogs), NA infusion 8 hours followed by saline infusion up to 24 hours (12 dogs), saline infusion 8 hours (6 dogs) and saline infusion 24 hours (5 dogs) The last two groups will be referred to as control animals

Blood samples on the average 10—15 ml, were drawn from a polyethylene catheter in the femoral artery every two hours The first sample was taken immediately before the start of the infusions Liver biopsies were made surgically through a midline incision, the pieces weighing around 300 mg each, and muscle biopsies similarly from the hind limb musculature at 0 8 and 24 hours In some animals samples were taken at 2 12, 16 or 24 hours as well At the end of each experiment, pieces from liver (anterior margin), gracilis muscle diaphragm (anterolateral part) heart (apex) lung (anterolateral margin of lower lobe) and kidney were taken After a small part of each sample had been removed for histological preparation, the specimens intended for biochemical analysis (liver heart and muscle) were quickly frozen in liquid nitrogen Sampling was completed within three minutes

In some of the animals observations on blood pressure, rectal temperature, respira

control dogs and in dogs receiving NA for 8 as well as for 24 hours Mean value±S E. of mean and

16	18	20	22	24
				0 82±0 12 n=5 <0 05
0 91±0 08 n=10 0 05	0 98±0 09 n=7 <0 01	0 98±0 09 n= 8 <0 01	1 00±0 11 n=7 <0 01	0 96±0 12 n=7 <0 05
2 23±0 25 n=3 <0 05	2 22±0 23 n=5 <0 01	2 24±0 24 n=5 <0 01	2 20±0 26 n=5 <0 01	1 57±0.27 n=5 <0 05
				0 18±0 03 n=2
0 23±0 06 n 5 0 05	0 22±0 06 n=4 0 05	0 24±0 04 n=4 >0 05	0 36±0 06 n=3 >0 05	0 39±0 20 n=2 >0 05
0 48 n 1	0 40 n=1	0 36 n=1		0 28 n=1
90 ±7 n 10 0 05	83±4 n 7 >0 05	86±4 n=8 >0 05	89±8 n 7 >0 05	83±7 n 4 >0 05
9 ±10 n 3 0 05	73±10 n=5 >0 05	70±12 n=5 0 05	69±11 n=5 >0 05	80 ±12 n=5 >0 05

Results

Physiological findings

The dogs infused with NA generally showed the following symptoms as compared to the control animals increased respiratory frequency (up to five times) and pulse rate and elevated body temperature (1 5 to 2 C) These symptoms appeared during the first hours and persisted throughout the infusion A moderate rise in blood pressure subsided within the first two hours after which the values were normal After withdrawal of NA the respiratory frequency decreased and in some cases returned to pre infusion levels The elevated body temperature persisted or fell only slowly The liver was yellow and friable and was felt to be warmer than the surrounding tissues also in animals where NA had been withdrawn for 12 hours or more

Of the 6 dogs which were to be infused with NA for 24 hours one died after 12 hours Six out of 12 dogs infused with NA for 8 hours and then with saline only died within 10—20 hours from the start of the experiment while the re

TABLE II Concentration of FFA and glycerol in plasma and glucose in blood at different times in number of animals analyzed (p as in table I)

		Time (hours)			
		0	10	12	14
FFA (mM/l)	Controls	0 43±0 09		0 95±0 14	
		n=5		n=5	
	p			<0 05	
	NA (8 hours)	0 67±0 06	1 13±0 10	0 95±0 07	0 89±0 06
		n=12	n=12	n=12	n=11
	p		<0 001	<0 01	<0 01
	NA (24 hours)	0 52±0 03	2 33±0 10	2 29±0 19	2 56±0 12
		n=6	n=6	n=6	n=4
	p		<0 001	<0 001	<0 001
Glycerol (mM/l)	Controls	0 07±0 01		0 18±0 04	
		n=2		n=2	
	NA (8 hours)	0 16±0 05	0 25±0 06	0 21±0 05	0 22±0 06
		n=5	n=5	n=5	n=5
	p	>0 05	>0 05	>0 05	>0 05
	NA (24 hours)	0 11	0 52	0 47	
		n=1	n=1	n=1	
Glucose (mg/100 ml)	NA (8 hours)	84±9	85±5	85±5	92±6
		n=12	n=12	n=12	n=11
	p	>0 05	>0 05	>0 05	>0 05
	NA (24 hours)	89±3	76±9	85±11	87±13
		n=5	n=6	n=6	n=5
	p	>0 05	>0 05	>0 05	>0 05

tral 10 % formalin, freeze sectioned on an ordinary freezing microtome and stained with Sudan III—IV in a mixture of 70 % ethanol and acetone with Ehrlich s or Harris' hematoxylin as counterstain

For histochemical studies small pieces from each sample were rapidly frozen in liquid nitrogen or in isopentane cooled with liquid nitrogen, and other pieces were fixed in ice cold formol sucrose solution for 24 hours and then kept in 0 32 M sucrose up to sectioning (35)

Serial sections were made at 7—15 μ in a cryostat (W Dittes, Heidelberg, Germany)

The *fresh frozen material* was stained as follows for succinic dehydrogenase with Nitro BT dissolved in N,N dimethylformamide as suggested by Pearson (52), for phosphorylase with Eränkö s and Palkama s modification (19) of the original method (61), for glycogen with periodic acid Schiff (PAS) staining in 70 % ethanol according to Mowry et al (45) Controls were made by incubation in 1 % diastase (Merck Darmstadt Germany) solution and in water alone for 1 hour at 20° C (24) before staining, for lipids with Sudan Black B in 70 % methanol (modified after Meier (44)) and for nonspecific esterase with α naphtyl acetate and Fast Blue B (51)

The *formol sucrose fixed material* was stained for glycogen, esterase and lipids and the sections from muscle as well for myoglobin with the benzidine-peroxidase method suggested by Drews and Engel (16)

had decreased They then continued to decrease and most so in the group infused for 24 hours

The lipid composition of the plasma *lipoproteins* is given in table V and their TG content in fig 3 There was a significant increase of all lipid components in the VLD at 24 hours, which was most pronounced for the triglycerides No changes occurred in the LD, while the phospholipids had increased at 1—2 as well as 24 hours in the HD

The *liver lipids* are given in table VI In fig 4 the liver TG are shown The concentration of cholesterol and phospholipids had increased slightly after infusion of NA for 8 hours and the TG had augmented six to sevenfold In the dogs where NA infusion was continued up to 24 hours the hepatic TG showed a further increase Withdrawal of NA at 8 hours was followed by a slight but significant decrease in liver TG although at 24 hours the concentration was still well above the initial level The decrease of TG from 8 to 24 hours was 12 ± 4 ($p < 0.05$) µmoles/g No significant changes occurred in the liver lipids of control dogs

The TG values of biopsy specimens from myocardium and skeletal muscle showed great fluctuations with variations of the TG content in skeletal muscle between 10 and 150 µmoles/g This was presumably due to the varying amounts of interstitial fat as revealed by the microscopic picture and will therefore not be considered further No significant changes in the phospholipid content were noted in these organs

Fig 5 shows the significant positive correlation between the increase in

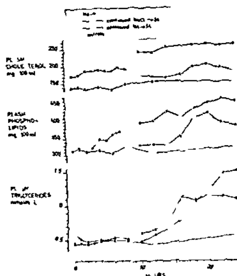


Fig 2 The concentration of cholesterol phospholipids and triglycerides in plasma in control dogs and in dogs receiving continuous infusion of NA for 8 and for 24 hours.

hepatic TG from 0 to 8 hours and the increase in plasma TG from 0 to 24 hours This correlation apparently was the same whether NA was given for 8 or 24 hours There was no significant correlation between the FFA levels in plasma and the increase in hepatic TG at 8 hours

Histological findings

Liver Sudan positive lipid was found in single fat cells only and as droplets in the biliary epithelium at the beginning of the experiments and in the controls from 0 to 24 hours After 2 hours of NA infusion small droplets appeared in the sinusoidal border of periportal cells and in some of the pericentral ones After 8 hours there were fat droplets in practically all liver cells In most cases the droplet size and density decreased from the periportal areas towards the central

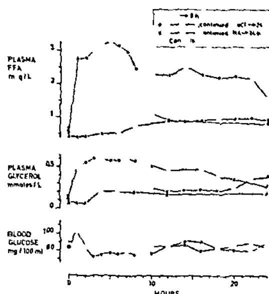


Fig 1 The concentration of FFA and glycerol in plasma and of glucose in blood in control dogs and in dogs receiving continuous infusion of NA for 8 and for 24 hours

maining 6 lived up to sacrification at 24 hours Death was preceded by deteriorated general condition, which was characteristic also of the surviving animals in this group Small subserosal petechiae, especially in the intestines, were found in some of the animals that died before the end of the experiment and as well in some of those infused with NA up to 24 hours

Biochemical findings

The concentrations of FFA glycerol and glucose are given in tables I and II and fig 1 The *FFA concentration* increased slightly in the control animals and significantly during the infusion of NA in the experimental animals In those dogs where NA was withdrawn after 8 hours, the FFA levels rapidly decreased and became the same as in the controls The *glycerol concentration* in plasma behaved in a way similar to that of FFA during the infusion of NA After withdrawal of the infusion, however, the values remained slightly increased The *blood glucose level* increased significantly after infusion of NA for 1 hour and then dropped below the fasting level at 3 hours After that time no significant changes were observed

The concentration of cholesterol, phospholipids, triglycerides and proteins in plasma are given in tables III and IV and in fig 2 The *concentration of cholesterol and phospholipids* increased during the first two hours of NA infusion In the control dogs only the phospholipids increased At 24 hours the concentration of cholesterol and phospholipids was significantly raised in the two NA treated groups In the controls, only the phospholipids were significantly increased There was no statistically significant difference between the *increases* in the concentration of phospholipid in treated and control dogs The *plasma TG* had decreased slightly in the control group and had increased slightly in the NA groups at 6 hours At about 12 hours, the TG started to increase more rapidly in the two NA groups At 24 hours, the TG level had increased about three times The control dogs showed no significant change The concentration changes in the lipid fractions, except for that of cholesterol in the 24-hour NA group are still significant even when no corrections are made for changes in plasma protein concentration

The *plasma protein concentration* showed no changes in the control group NA induced an increase of the plasma proteins during the first two hours of infusion After 6 hours the plasma proteins

of control dogs and of dogs receiving NA for 8 hours. Mean value ± S.E. of mean and number of

4	5	6	7	8
141±16 n=11 >0.05		135±17 n=10 >0.05		152±17 n=11 >0.05
190±15 n=15 <0.001	189±15 n=20 <0.001	189±16 n=19 <0.001	197±17 n=20 <0.001	193±15 n=20 <0.001
315±26 n=11 0.05		304±28 n=10 >0.05		330±29 n=11 >0.05
346±23 n=23 0.001	346±23 n=22 <0.001	362±26 n 23 <0.001	370±26 n=22 <0.001	355±24 n=22 <0.001
0.51±0.08 n 11 0.05		0.50±0.11 n=10 <0.05		0.49±0.06 n=11 <0.05
0.48±0.04 n 23 0.05	0.51±0.05 n=22 0.05	0.54±0.05 n=22 <0.01	0.54±0.05 n=23 <0.01	0.51±0.05 n=23 0.05
5.7±0.2 n 11 0.05		5.6±0.2 n=10 >0.05		5.6±0.2 n 11 >0.05
5.6±0.1 n 22 0.05	5.5±0.1 n=22 >0.05	5.3±0.1 n=22 <0.01	5.2±0.1 n=22 0.001	5.2±0.1 n 22 <0.01

intensity in periportal areas. There were coarser formazan deposits in NA infused animals.

Skeletal muscle

Stainings for succinic dehydrogenase, phosphorylase and myoglobin confirmed the presence of three different fiber types in the dog as in other vertebrates (48, 49, 64): *red fibers* rich in myoglobin and succinic dehydrogenase but poor in phosphorylase, *white fibers* vice versa, and *intermediate fibers*. In the fresh frozen material esterase staining was generally strong in red fibers but in the material fixed in formol-sucrose the distribution was different as *presumably the same enzyme(s) were* not equally demonstrated in unfixed and fixed specimens.

Three different degrees in the deposition of Sudan positive lipid were also found throughout the material. As in general the content of such droplets

TABLE III. Concentration of cholesterol, phospholipids, TG and proteins at different times in plasma animals analyzed (p as in table I)

		Time (hours)			
		0	1	2	3
Cholesterol (mg/100 ml)	Controls	141±15 n=11	142±17 n=11	134±16 n=10	
	p		>0.05	>0.05	
	NA	174±14 n=20	175±13 n=20	188±14 n=19	190±16 n=19
	p		>0.05	<0.01	<0.001
Phospholipids (mg/100 ml)	Controls	301±23 n=11	320±26 n=11	311±25 n=10	
	p		<0.01	<0.001	
	NA	315±22 n=22	322±21 n=23	342±21 n=22	323±28 n=22
	p		<0.05	<0.001	<0.001
TG (mM/l)	Controls	0.59±0.12 n=11	0.51±0.08 n=11	0.48±0.07 n=10	
	p		>0.05	0.05	
	NA	0.44±0.04 n=22	0.45±0.04 n=23	0.44±0.04 n=22	0.46±0.04 n=22
	p		>0.05	>0.05	>0.05
Proteins (g/100 ml)	Controls	5.7±0.2 n=11	5.7±0.2 n=11	5.5±0.2 n=10	
	p		0.05	>0.05	
	NA	5.6±0.04 n=22	5.8±0.1 n=22	5.8±0.1 n=22	5.6±0.1 n=21
	p		<0.001	0.01	<0.05

veins. After 24 hours fat was uniformly distributed throughout the liver lobules. Although some cells showed signs of cytoplasmic damage with confluence of fat droplets and displacement of nuclei, most of the cells had retained their fundamental structure but for the great number of fat droplets filling the cytoplasm. In the dogs given NA for the first 8 hours only of a 24-hour experiment, there was at the end a more uniform distribution of fat droplets in the lobules than at 8 hours and in a few cases there was even less fat in periportal cells than in those around central veins.

Glycogen was found mainly in central areas at the beginning of the experiments, in later biopsy specimens only single cells as a rule contained demonstrable amounts.

Stainings for succinic dehydrogenase and nonspecific esterase showed a fairly even distribution with somewhat higher

1

of control dogs and of dogs receiving NA for 8 hours Mean value ± S E of mean and number of

4	5	6	7	8
141±16 n=11 >0 05		135±17 n=10 >0 05		152±17 n=11 >0 05
190±15 n=15 <0 001	189±15 n=20 <0 001	189±16 n=19 <0 001	197±17 n=20 <0 001	193±15 n=20 <0 001
315±26 n=11 0 05		304±28 n=10 >0 05		330±29 n=11 >0 05
346±23 n 23 0 001	346±23 n=22 <0 001	362±26 n 23 <0 001	370±26 n=22 <0 001	355±24 n 22 <0 001
0 51±0 08 n 11 >0 05		0 50±0 11 n 10 <0 05		0 49±0 06 n=11 <0 05
0 48±0 04 n=23 0 05	0 51±0 05 n=22 >0 05	0 54±0 05 n=22 <0 01	0 54±0 05 n 23 <0 01	0 51±0 05 n 23 >0 05
5 7±0 2 n=11 >0 05		5 6±0 2 n=10 >0 05		5 6±0 2 n=11 >0 05
5 6±0 1 n 22 >0 05	5 5±0 1 n=22 >0 05	5 3±0 1 n=22 <0 01	5 2±0 1 n 22 <0 001	5 2±0 1 n=22 <0 01

intensity in periportal areas There were coarser formazan deposits in NA infused animals

Skeletal muscle

Stainings for succinic dehydrogenase phosphorylase and myoglobin confirmed the presence of three different fiber types in the dog as in other vertebrates (48 49 64) *red' fibers* rich in myo globin and succinic dehydrogenase but poor in phosphorylase *white fibers* vice versa and *intermediate fibers* In the fresh frozen material esterase staining was generally strong in red fibers, but in the material fixed in formol sucrose the distribution was different as presumably the same enzyme(s) were not equally demonstrated in unfixed and fixed specimens

Three different degrees in the deposi tion of Sudan positive lipid were also found throughout the material As, in general the content of such droplets

TABLE III Concentration of cholesterol, phospholipids, TG and proteins at different times in plasma animals analyzed (p as in table I)

		Time (hours)			
		0	1	2	3
Cholesterol (mg/100 ml)	Controls	141±15	142±17	134±16	
		n=11	n=11	n=10	
	p		>0 05	>0 05	
	NA	174±14	175±13	188±14	190±16
		n=20	n=20	n=19	n=19
	p		>0 05	<0 01	<0 001
Phospholipids (mg/100 ml)	Controls	301±23	320±26	311±25	
		n=11	n=11	n=10	
	p		<0 01	<0 001	
	NA	315±22	322±21	342±21	323±28
		n=22	n=23	n=22	n=22
	p		<0 05	<0 001	<0 001
TG (mM/l)	Controls	0 59±0 12	0 51±0 08	0 48±0 07	
		n=11	n=11	n=10	
	p		>0 05	>0 05	
	NA	0 44±0 04	0 45±0 04	0 44±0 04	0 46±0 04
		n=22	n=23	n=22	n=22
	p		>0 05	>0 05	>0 05
Proteins (g/100 ml)	Controls	5 7±0 2	5 7±0 2	5 5±0 2	
		n=11	n=11	n=10	
	p		>0 05	>0 05	
	NA	5 6±0 04	5 8±0 1	5 8±0 1	5 6±0 1
		n=22	n=22	n=22	n=21
	p		<0 001	<0 01	>0 05

veins After 24 hours fat was uniformly distributed throughout the liver lobules Although some cells showed signs of cytoplasmic damage with confluence of fat droplets and displacement of nuclei, most of the cells had retained their fundamental structure but for the great number of fat droplets filling the cytoplasm In the dogs given NA for the first 8 hours *only* of a 24-hour experiment, there was at the end a more uniform distribution of fat droplets in the lobules than at 8 hours, and in a few cases there was even less fat in periportal cells than in those around central veins

Glycogen was found mainly in central areas at the beginning of the experiments, in later biopsy specimens only single cells, as a rule, contained demonstrable amounts

Stainings for succinic dehydrogenase and nonspecific esterase showed a fairly even distribution with somewhat higher

of control dogs and of dogs receiving NA for 8 as well as for 24 hours Mean value ± S.E. of mean

16	18	20	22	24
				154±19 n=6 >0.05
175±19 n 9 <0.01	198±24 n=7 <0.01	203±25 n=7 <0.01	196±24 n=6 <0.01	185±24 n=6 <0.05
	263±46 n=3 <0.01	263±54 n=3 >0.05	260±51 n=3 <0.05	264±54 n=3 <0.05
				325±38 n=6 <0.05
356±41 n=10 <0.001	411±58 n=7 <0.01	422±51 n=8 <0.01	396±47 n=7 <0.01	387±45 n=7 <0.01
412±85 n=3 <0.05	437±49 n=5 <0.001	452±59 n=5 <0.01	465±58 n=5 <0.01	458±53 n=5 <0.01
				0.58±0.15 n=6 >0.05
0.81±0.18 n 9 <0.05	1.09±0.23 n=7 <0.05	1.15±0.22 n=8 <0.01	1.14±0.20 n=7 <0.01	1.10±0.16 n=7 <0.01
1.14±0.25 n=3 <0.05	1.08±0.24 n=5 <0.05	1.25±0.24 n=5 <0.05	1.47±0.24 n=5 <0.01	1.51±0.27 n=5 <0.01
				5.7±0.2 n=6 >0.05
5.0±0.2 n 8 <0.05	5.1±0.2 n=8 <0.05	5.1±0.2 n=8 <0.01	5.1±0.2 n=7 <0.05	5.2±0.2 n=7 <0.05
4.4±0.3 n 5 <0.05	4.2±0.4 n=3 >0.05	4.3±0.3 n=5 <0.01	4.2±0.3 n=5 <0.05	4.2±0.3 n=5 <0.05

Small Sudan positive droplets were found in single red and intermediate fibers in rows between myofibrils, at the beginning of the experiments and in the controls from 0 to 24 hours At 2 and 4 hours of infusion no appreciable

TABLE IV Concentration of cholesterol, phospholipids, TG and proteins at different times in plasma and number of animals analyzed (p as in table I)

		Time (hours)			
		0	10	12	14
Cholesterol (mg/100 ml)	Controls	150±19 n=5		158±23 n=5	
	p			>0 05	
	NA (8 hours)	155±18 n=12	175±19 n=12	170±18 n=11	167±20 n=10
	p		<0 01	<0 01	<0 01
	NA (24 hours)	216±29 n=4	244±26 n=4	241±29 n=4	256±43 n=3
	p		<0 05	>0 05	<0 01
Phospholipids (mg/100 ml)	Controls	270±37 n=5		304±48 n=5	
	p			>0 05	
	NA (8 hours)	285±31 n=12	330±34 n=12	332±34 n=12	330±34 n=11
	p		<0 01	<0 01	<0 001
	NA (24 hours)	352±108 n=6	396±39 n=6	400±37 n=6	429±54 n=4
	p		<0 05	<0 05	<0 05
TG (mM/l)	Controls	0 61±0 08 n=5		0 43±0 09 n=5	
	p			>0 05	
	NA (8 hours)	0 38±0 03 n=11	0 48±0 06 n=12	0 57±0 09 n=11	0 67±0 11 n=11
	p		>0 05	>0 05	>0 05
	NA (24 hours)	0 45±0 05 n=6	0 60±0 10 n=6	0 65±0 16 n=6	0 77±0 23 n=4
	p		>0 05	>0 05	>0 05
Proteins (g/100 ml)	Controls	6 1±0 4 n=5		5 9±0 2 n=5	
	p			>0 05	
	NA (8 hours)	5 6±0 1 n=12	5 0±0 2 n=12	5 2±0 2 n=11	5 1±0 2 n=10
	p		<0 001	<0 01	<0 01
	NA (24 hours)	5 6±0 2 n=6	4 8±0 2 n=6	4 6±0 2 n=6	4 4±0 3 n=4
	p		<0 05	<0 01	<0 05

was related to that of myoglobin, the fibers in the freeze sectioned Sudan III—IV stained biopsy specimens will for practical reasons be referred to as "red", "white" and "intermediate" according to their content of fat droplets

of control dogs and of dogs receiving NA for 8 as well as for 24 hours Mean value ± S E of mean

16	18	20	22	24
				154±19 n 6 >0 05
175±19 n=9 <0 01	198±24 n=7 <0 01	203±25 n=7 <0 01	196±24 n 6 <0 01	185±24 n–6 <0 05
	263±46 n=3 <0 01	263±54 n=3 >0 05	260±51 n=3 <0 05	264±54 n=3 <0 05
				325±38 n=6 <0 05
350±41 n 10 <0 001	411±58 n=7 <0 01	422±51 n=8 <0 01	396±47 n=7 <0 01	387±45 n=7 <0 01
412±85 n 3 <0 05	437±49 n–5 <0 001	452±59 n=5 <0 01	465±58 n=5 <0 01	458±53 n 5 <0 01
				0 58±0 15 n 6 >0 05
0 81±0 18 n–9 <0 05	1 09±0 23 n=7 <0 05	1 15±0 22 n=8 <0 01	1 14±0 20 n=7 <0 01	1 10±0 16 n 7 <0 01
1 14±0 25 n–3 <0 05	1 08±0 24 n=5 <0 05	1 25±0 24 n=5 <0 05	1 47±0 24 n=5 <0 01	1 51±0 27 n=5 <0 01
				5 7±0 2 n 6 >0 05
5 0±0 2 n 8 0 05	5 1±0 2 n=8 <0 05	5 1±0 2 n–8 <0 01	5 1±0 2 n=7 <0 05	5 2±0 2 n=7 <0 05
4 4 0 3 n 5 <0 05	4 2±0 4 n=3 >0 05	4 3±0 3 n 5 <0 01	4 2±0 3 n=5 <0 05	4 2±0 3 n–5 <0 05

Small Sudan positive droplets were found in single red and intermediate fibers in rows between myofibrils, at the beginning of the experiments and in the controls from 0 to 24 hours At 2 and 4 hours of *infusion no appreciable*

TABLE IV. Concentration of cholesterol, phospholipids, TG and proteins at different times in plasma and number of animals analyzed (p as in table I)

		Time (hours)			
		0	10	12	14
Cholesterol (mg/100 ml)	Controls	150±19		158±23	
		n=5		n=5	
	p			>0.05	
	NA	155±18	175±19	170±18	167±20
	(8 hours)	n=12	n=12	n=11	n=10
	p		<0.01	<0.01	<0.01
	NA	216±29	244±26	241±29	256±43
	(24 hours)	n=4	n=4	n=4	n=3
	p		<0.05	>0.05	<0.01
Phospholipids (mg/100 ml)	Controls	270±37		304±48	
		n=5		n=5	
	p			>0.05	
	NA	285±31	330±34	332±34	330±34
	(8 hours)	n=12	n=12	n=12	n=11
	p		<0.01	<0.01	<0.001
	NA	352±108	396±39	400±37	429±54
	(24 hours)	n=6	n=6	n=6	n=4
	p		<0.05	<0.05	<0.05
TG (mM/l)	Controls	0.61±0.08		0.43±0.09	
		n=5		n=5	
	p			>0.05	
	NA	0.38±0.03	0.48±0.06	0.57±0.09	0.67±0.11
	(8 hours)	n=11	n=12	n=11	n=11
	p		>0.05	>0.05	>0.05
	NA	0.45±0.05	0.60±0.10	0.65±0.16	0.77±0.23
	(24 hours)	n=6	n=6	n=6	n=4
	p		>0.05	>0.05	>0.05
Proteins (g/100 ml)	Controls	6.1±0.4		5.9±0.2	
		n=5		n=5	
	p			>0.05	
	NA	5.6±0.1	5.0±0.2	5.2±0.2	5.1±0.2
	(8 hours)	n=12	n=12	n=11	n=10
	p		<0.001	<0.01	<0.01
	NA	5.6±0.2	4.8±0.2	4.6±0.2	4.4±0.3
	(24 hours)	n=6	n=6	n=6	n=4
	p		<0.05	<0.01	<0.05

was related to that of myoglobin, the fibers in the freeze sectioned Sudan III—IV stained biopsy specimens will for practical reasons be referred to as "red", "white" and "intermediate" according to their content of fat droplets.

TABLE VI Concentration of cholesterol, phospholipids and triglycerides in the liver of control dogs and of dogs receiving NA for 8 as well as for 24 hours. Mean value ± S.E. of mean and number of animals analyzed (p as in table I)

	Time (hours)					
	0	8		24		
					NA for	
Group		*Controls*	NA	*Controls*	*8 hours*	*24 hours*
Cholesterol (mg/g)	3.5±0.2 n=16	3.0±0.2 n=6	3.9±0.2 n=18	3.1±0.5 n=5	4.1±0.3 n=5	4.3±0.4 n=3
p		>0.05	<0.01	>0.05	>0.05	>0.05
Phospholipids (mg/g)	24.2±1.7 n=20	27.1±1.8 n=6	27.2±1.2 n=23	26.1±0.7 n=5	24.5±1.8 n=5	24.0±3.3 n=5
p		>0.05	<0.001	>0.05	>0.05	>0.05
TG (μM/g)	6.9±1.1 n=20	18.5±9.7 n=6	46.6±4.1 n=23	15.8±5.0 n=5	37.0±6.5 n=5	108.4±13.5 n=5
p		>0.05	<0.001	>0.05	<0.01	<0.01

to 8 hours after the withdrawal of the NA infusion and within 24 hours after the start of the experiment the initial appearance was restored or there were even less fat droplets than before the infusion (fig. 6C—F). Many fibers looked edematous at this stage. In the controls the fat content was essentially unchanged. Glycogen was generally found in amounts corresponding to the phosphorylase content of each fiber type, i.e. most in the white fibers and least in the red. Except for a slight decrease in demonstrable glycogen in red fibers no significant changes were seen. The distribution and intensity of esterase and phosphorylase staining remained essentially unaltered. The reaction for succinic dehydrogenase was intensified in NA infused animals where the formazan deposits were darker and somewhat larger than before the start of the infusion or in control animals. The usual longitudinal arrangement of formazan deposits was not as regular in muscle fibers of NA-infused animals. This change persisted after withdrawal of NA.

Heart

Sudan positive droplets, probably lipofuscin, were seen in perinuclear spaces in untreated and control animals, but none were found in the sarcoplasm between myofibrils. After 8 hours and more so after 24 hours of NA infusion minute fat droplets, the size and position similar to those of mitochondria were seen in practically all myocardial fibers. Twenty four hours after the beginning of an 8-hour infusion the myocardial fibers were slightly edematous but except for occasional lipofuscin granules only focal aggregations of lipid were observed and in most specimens no fat

TABLE V Concentration at 0 hours and changes in concentration from 0 hours of cholesterol, phospholipids and TG in different plasma protein classes in dogs receiving NA for 8 or 24 hours. Mean value ± S E of mean and number of animals analyzed (p as in table I)

Lipoprotein class	Lipid component	Concentration at 0 hours	Change in concentration from 0 to 1—2 hours	Change in concentration from 0 to 24 hours
Very low density	Cholesterol (mg/100 ml)	2 4±5 5 n=8	−1 5±0 9 n=6	7 5±2 9 n=6
	p		>0 05	<0 05
	Phospholipids (mg/100 ml)	5 4±1 7 n=8	−0 2±0 3 n=6	19 0±6 2 n=6
	p		>0 05	<0 05
	TG (mM/l)	0 13±0 04 n=8	−0 01±0 01 n=6	0 70±0 21 n=6
	p		>0 05	<0 05
Low density	Cholesterol (mg/100 ml)	16±4 n=7	0±2 =5	−1±3 n=5
	p		>0 05	>0 05
	Phospholipids (mg/100 ml)	28±3 n=7	3±1 n=5	7±4 n=5
	p		>0 05	>0 05
	TG (mM/l)	0 22±0 04 n=7	−0 03±0 02 n=5	0 00±0 04 n=5
	p		>0 05	>0 05
High density	Cholesterol (mg/100 ml)	128±12 n=8	−1±1 n=6	6±3 n=6
	p		>0 05	>0 05
	Phospholipids (mg/100 ml)	245±24 n=8	15±5 n=6	48±18 n=6
	p		>0 05	<0 05
	TG (mM/l)	0 04±0 01 n=8	−0 01±0 01 n=6	−0 01±0 01 n=6
	p		>0 05	>0 05

changes had occurred but at 8 hours differences between fibers were easily noticeable as red fibers were abundantly and intermediate moderately filled with fat droplets, whereas only single droplets were seen in white fibers (figs 6 A—B) After 24 hours of NA infusion the cross striation of red fibers was often masked by considerable amounts of fat droplets which seemed to occupy all free space in the sarcoplasm Less droplets were seen in intermediate fibers, and white fibers contained only few (fig 7 A) The diaphragmal fibers were not as affected as those from the thigh musculature In the dogs infused with NA for the first 8 hours only, reduction of the stainable lipid content was observed already at 4

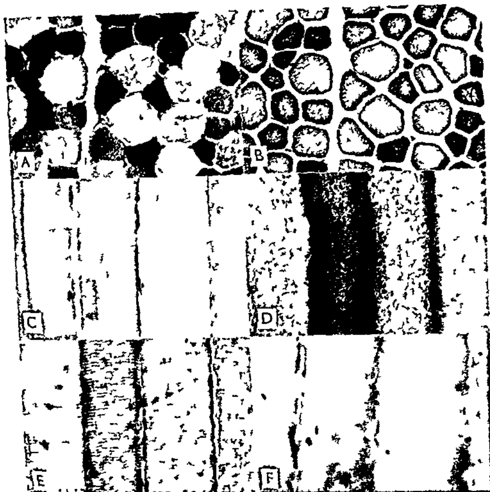


Fig F A Cross sect on f om grac lis muscle of dog af er 8 hours of NA nfus on. Benz dine peroxidase reac ion for myoglobin The d fference in myoglob n ontent between f bers s dem ons a ed 120 B Ad acent se tion stained for l p d w h *Sudan Black B* The myoglobin rich ed fibers sh w a l gh content of sta ned l p d Bo h sect ons a e sl ghtly obl que 120 C F Longi ud nal sections *from* grac lis muscle of dog rece ing NA infus on from 0 to 8 hours of a 16-hour per od Formalin *fixation freeze* section ng and sta ning w h Sudan III IV and hematoxyl n Distr bution and amount of fat d oplets at 0 C 8 (D 12 E and 16 F hours N *e the d fference n l p d con ent between fiber types of A and B 300 × The f bers n F are* sl gh ly edematous.

my f l r ls In all infused an mals the formazan deposits were coarser than in controls and parted so that tl eir distribu t on followed rather tl e *transverse* stria tions Tl s change was seen un formly tl roughout the specimens

Lung

Occas onal fat doplets were seen in alveolar wall cells [type II of Karrer 37) Nagaishi et al (46)] of untreated *animals* In infused dogs especially after 24 hours a great number of fat

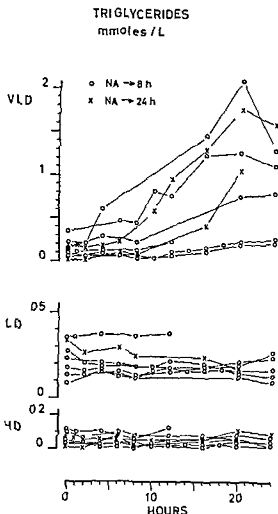


Fig 3 The concentration of triglycerides in very low density (VLD), low (LD) and high density (HD) lipoproteins in dogs receiving continuous infusion of NA for 8 and for 24 hours

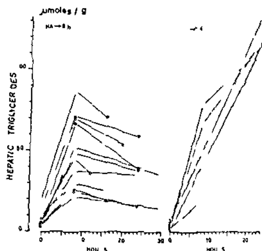


Fig 4 The concentration of triglycerides in the livers of dogs receiving continuous infusion of NA for 8 hours (left part) and for 24 hours (right part)

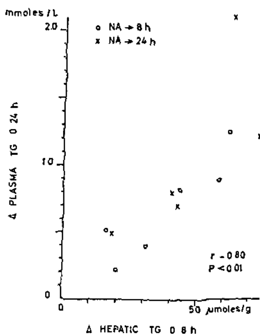


Fig 5 The relationship between the increase in plasma triglycerides from 0 to 24 hours and the increase in hepatic triglycerides from 0 to 8 hours in dogs receiving continuous infusion of NA for 8 and for 24 hours

droplets could be detected No significant changes were seen in the controls

*Glycogen in small amounts was dem*onstrable mainly in perinuclear spaces in 8-hour and control animals In dogs infused with NA for 24 hours there was an increase in the amount of stainable glycogen also at the cross striations

Nonspecific esterase and phosphorylase were uniformly distributed in the myocardium and did not show any significant changes in the experimental animals or controls In control animals, succinic dehydrogenase staining showed the usual arrangement of formazan deposits in longitudinal rows between

treated animals In all NA infused animals there was an increase in the amount of fat in this part of the nephron from 0 to 24 hours In specimens taken after 8 hours minute fat droplets were also seen in the proximal convolution and after 24 hours of infusion nearly all tubular epithelia except for the thin segment of Henle s loop contained stainable fat n b in the parts of the kidney that were at all affected In some parts of the renal pyramids fat free tubules were seen among those with lip d deposits Single cells in the vascular poles of glomeruli often displayed a distinct Sudan staining

Esterase activity was strong in the straight portion of the proximal tubules moderate in the proximal and distal convolutions and weak in glomerular cells Glycogen was demonstrable in scanty amounts in the collecting tubules but not in distal or proximal tubules (42) Strong succinic dehydrogenase activity was present in distal tubules and strong to moderate in proximal tubules In NA nfused animals coarser deposition of formazan occurred

Discussion

Plasma l p d changes and their relat on to intracellular depos t on of l pids

The augmented release of FFA from ad pose tissue induced by NA is reflected i rap dly rising plasma levels which eventually reach a steady state Isotopic stud es in dogs have sl own that during this steady state the turnover of FFA is increased 2 12) This impl es augmented tissue uptake of plasma FFA (12 60)

Tl e r se in plasma TG of NA infused dogs suggests an augmented production from liver cells which are stated to be the main source of plasma lipoproteins (3 26 29, 38 59) The primary cause of this increase is very probably the enhanced FFA mobilization since triglyceridemia is observed after administration of adipokinetic substances other than NA e g adrenaline (17) and pituitary preparations (55) The similarity between TG concentration curves from 0 to 24 hours in dogs infused with NA for 8 and 24 hours respectively is noteworthy It suggests that the FFA mobilized during the first 8 hours are the primary determinant of the plasma TG concentration at 24 hours This is further supported by the fact that the 24-hour increase in plasma TG is directly correlated to the increase in liver TG from 0 to 8 hours Moreover there is the same linear relationship between the plasma TG concentration at 24 hours and the liver TG content at 8 hours in both groups

The increase in plasma TG was entirely confined to VLD lipoproteins in accordance with the findings of Havel (27) It is however of considerable interest that while the peak concentration of label in plasma TG appears within one hour after injection of labeled FFA in the dog (29 Boberg and Carlson unpublished) the concentration of plasma TG in the present series does not rise markedly until several hours after stimulation of FFA turnover by NA infusion

The present results show that an increase in the levels of plasma FFA is the primary determinant of intracellular deposition of lip ds during the experi

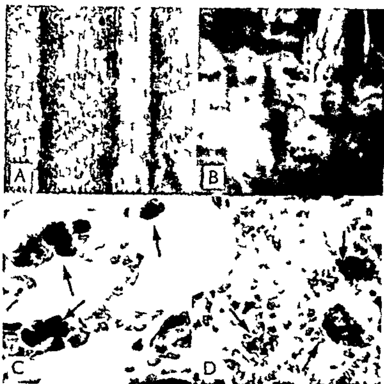


Fig 7 A Longitudinal section from gracilis muscle of dog after 24 hours of NA infusion Abundant fat droplets especially in red fibers Same preparation method as in fig 6 C—F 300× B Oblique section through the wall of lung artery in a dog receiving NA infusion for 24 hours. Small fat droplets (black dots) in the cytoplasm of smooth muscle cells Same preparation method 1 200× C and D Slightly collapsed lung alveoles and atelectatic area of lung from dog after 24 hours of NA infusion Heavy lipid deposition in alveolar wall cells and macrophages (arrows) Same preparation method 300×

laden alveolar wall cells and macrophages occurred After a 24 hour infusion there were many small atelectases *in the parenchyma and fat laden* cells were often found in clusters in the atelectatic areas (fig 7C—D) The bronchiolar epithelium showed an abundance of fat droplets mainly in the apical parts of the cells In the smooth muscle cells of arteries and bronchioles small fat droplets occurred in the perinuclear spaces (fig 7B) Free alveolar wall cells filled with fat were seen in alveolar and bronchiolar lumina often nearly occluding the air passages In the 8 hour group practically no lipid deposits and only occasional atelectases were demonstrable after 24 hours In 24-hour controls occasional atelectases with fat laden alveolar wall cells and *macrophages were seen Otherwise the* lungs of control animals showed a normal appearance

Esterase activity was strong in bronchial and bronchiolar epithelia and in alveolar wall cells Scanty amounts of glycogen were also seen in these locations A few fat emboli were observed in animals from all groups

Kidney

Fat droplets of varying size up to 3—4 μ were occasionally found in some cells of the proximal straight segment in un

product of this metabolism has been recognized Morphological evidence points to the aforementioned alveolar wall cells as being the source of this lining film (46) This concept is supported by the present findings which show the gradual increase in their lipid content during FFA mobilization Lipid is also eliminated by the lung through the detachment of fat laden alveolar wall cells and macrophages which are normally found in the sputum (53), a kind of holocrine secretion as it were

Oxidation of fat is stated to be the main energy source for heart, skeletal muscle and kidney in the fasting state These organs all take up considerable amounts of FFA (23) In working skeletal muscle and myocardium oxidation is an effective mean of eliminating intra cellular fat Diaphragmal fibers do not accumulate lipid to the same extent as those in the resting limb muscle Withdrawal of NA infusion reveals however that lipid may be effectively eliminated also from resting muscle, as at the end of the experiment in these animals, the specimens from limb muscle contain even less fat droplets than before the infusion was started As is suggested by the glycerol values lipolysis proceeds also in other tissues than the adipose tissue In skeletal muscle however hydrolyzed fatty acids are not released to plasma but are oxidized by mitochondrial enzymes Again the importance of the intracellular fat pool in determining the rate and extent of lipid depletion comes into the mind

The overall increase in formazan deposition in the reaction for succinic dehydrogenase may be a sign of enhanced oxidative activity in otherwise normal mitochondria It is not likely to be attributable to mitochondrial damage, since the ultrastructure is not conspicuously affected (42)

During enhanced FFA mobilization, fat is preferred to carbohydrates as energy source as the respiratory quotient falls (31) The increase in glycogen content of myocardial fibers both in NA infused and control animals at 24 hours may be explained by this preferential oxidation of fat instead of carbohydrates (33)

Physiological implications

Elevated body temperature, increased respiratory frequency decreased plasma protein and a transient rise in blood pressure were the clinical symptoms common for most animals infused with NA However only the effect on blood pressure can be attributed specifically to NA The decrease in plasma proteins suggesting a loss in blood volume, may be due in part to the vascular effects of the NA infusion (40), though the possible effects of FFA hypermobilization should also be considered The thermogenesis and increased oxygen consumption may be at least partly ascribed to the mobilization of FFA (60) This concept is further supported by recent experiments which show that the NA induced rise in oxygen consumption and also in temperature may be prevented by suppression of plasma FFA levels with nicotinic acid (31) or glucose infusion (Carlson Liljedahl and Wirsén unpublished)

The elevated body temperature decreases only gradually after withdrawal

mental period, whereas the developing triglyceridemia does not appear to contribute significantly

Uptake and storage of mobilized FFA

The efflux of FFA from plasma to tissue cells is believed to be dependent on the plasma concentration (21, 43, 54, 58), or on that of the medium in *in vitro* experiments (18) Whereas this may well be true for the total uptake of FFA as measured biochemically, the distribution of sudanophilic droplets in histochemically heterogeneous organs such as muscle or kidney suggests that, at the cellular level, as well inherent metabolic qualities as reflected in esterase activity or myoglobin content may determine the amounts taken up and esterified

Admittedly, differences in the number of fat droplets do not *per se* prove a selective uptake, since the FFA taken up may also have been incorporated in compounds not visualized as droplets, e g phospholipids It has been shown by autoradiography, however, that in pigeon breast muscle the small red fibers display the strongest radioactivity after intravenous injection of albumin bound labeled palmitic acid (65) As the red fibers also contain most fat droplets, we may assume that, at least in skeletal muscle, the staining used will give an adequate picture of the distribution of cellular uptake and esterification of FFA The premise of this assumption is, evidently, that elimination of incorporated radioactive FFA is insignificant as compared to the flux into the muscle fibers (65)

The fat droplets represent, in all probability, the intracellular stores of TG The storage in this form of fatty acids is to be regarded not only as the result of a temporary overflow, but as a physiologic process Recent studies in muscle metabolism (30, 36, 47) reveal that the intracellular fat pool may be no less important as immediate energy source than FFA taken up from plasma (12, 15) It has been possible to follow in the present series the growth and localization of this intracellular pool in muscle as well as in other tissues It is noteworthy that the largest stores are deposited in cells adapted to using fat as fuel or otherwise specifically involved in lipid metabolism

Depletion of intracellular lipid stores

The amount of intracellular lipid decreases after withdrawal of NA infusion, as can be seen both biochemically and morphologically Two main pathways for such a depletion are conceivable firstly, secretion of the fatty acids in esterified form, e g in lipoproteins, secondly, oxidation of the fatty acids by mitochondrial enzymes

Part of the fatty acids taken up and esterified by liver parenchymal cells are coupled to lipoproteins and recirculated in the blood As indicated by the present data, this process is accelerated — maybe chiefly — by the increase in the intracellular fat pool

The lung has long been known to take part in the lipid metabolism of the body (34, 53) With the discovery of the surface-active alveolar lining film (50) which is necessary to ensure an even inflation of alveoli (1 13), and the identification of its main constituent as dipalmityl lecithin (5) an essential end

from about 12 *hours onwards* The TG content of the liver increased markedly during the *infusion* but decreased slightly between 8 and 24 hours in the 8-hour group The similarity in the two groups as regards plasma TG levels may be explained by the significant positive correlation between the increase in liver TG from 0 to 8 hours and the total increase in plasma TG from 0 to 24 hours, suggesting that FFA uptake in the liver during the first 8 hours determines plasma TG levels during the experimental period Sudan positive droplets appeared in the liver, skeletal musculature — significantly more in red fibers than in intermediate and white —, myocardium bronchiolar epithelium and alveolar wall cells of the lung and in the proximal tubules of the kidney Their number increased during the NA infusion but decreased markedly between 8 and 24 hours in the 8-hour group except in the proximal tubules A transient rise in blood pressure was noted during the first hours as well as a slight increase in blood glucose levels There was a significant decrease in plasma protein in all NA infused animals The heart rate, respiratory frequency and rectal temperature increased during NA infusion and returned only slowly, if at all, towards normal levels after withdrawal It is suggested that the uptake of FFA from plasma in various tissue cells is a selective process and that changes in the size of the intracellular fat pool are of major importance in determining the rate of fatty acid oxidation or coupling to plasma lipoproteins

Acknowledgements

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of NA, however, and the liver parenchyma is still very warm for several hours. This lends support to the concept that high FFA levels are not the only determining factor in the clinical picture. As pointed out before, the enormous increase of the intracellular fat pool appears to be of major importance.

The atelectases seen after a 24-hour infusion may be due to impaired formation of the alveolar lining film. Excessive FFA flux into alveolar wall cells may constitute a serious overload, and the enhanced detachment of fat-laden such cells may deprive the lung of the necessary amount. The ensuing decrease in oxygenation may lead to a vicious circle as the alveolar wall cells presumably depend on a certain oxygen tension to be able to secrete the lining layer. This may in turn explain why fat-laden alveolar wall cells are also seen in poorly aerated lung tissue of control animals.

A similar syndrome to the one now described was observed by Woods and Kellner (66) in lean and obese rabbits given corticotropin. A marked rise in plasma FFA ensued, especially in the obese animals, which did not survive this "fatty acid intoxication". Heald and Rookledge (32) administered avian pituitary powder to rabbits and hens and noted that the rabbits were all severely affected, with FFA levels raised up to twenty times, whereas the hens did not show any reaction whatever. This confirms the recent observations of the extreme sensitivity of the rabbit to the adipokinetic action of pituitary preparations (55, 56) in contrast to the unresponsiveness of the domestic fowl in this respect (9).

It is evident that excessive release of FFA from the adipose tissue is a primary event in the syndromes now described. However, it does not seem altogether justified to restrict the condition to a mere "fatty acid intoxication". As the name implies, the symptoms would then be related to a toxic action of the fatty acids as such. Admittedly, very high FFA levels in plasma may be noxious. So may also excessive flux into cells, should there be an accumulation of yet unesterified fatty acids. But the present experiments suggest that the major problem is not esterification — i.e. "detoxification" — of FFA, but elimination of apparently overloaded stores. Until further information can be obtained about the mechanisms involved, we would therefore suggest the name "*lipid mobilization syndrome*". The high mortality and the deteriorated general condition of the animals after withdrawal of the NA infusion make it highly probable that not only the excessive mobilization of FFA, but also the various metabolic processes initiated by it, are deleterious to vital functions.

Summary

Continuous infusion of noradrenaline (NA) was given to anesthetized dogs during 8 and 24 hours respectively of a 24-hour experimental period. The levels of plasma free fatty acids (FFA) increased rapidly up to six-fold but returned promptly to baseline levels on withdrawal of the infusion. In both experimental groups there was a continuous increase in plasma triglycerides (TG), present in very low density lipoproteins,

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Obliterative Arterial Disease of the Lower Limbs

II A Study of the Course of the Disease

By

CARL TILLGREN

In order to evaluate the effects of long term anticoagulant treatment of patients with obliterative arterial disease in the lower limbs during the 1950s data from patients in Stockholm during this period were collected and analysed The material comprised treated' and untreated patients of various ages and with wide variations in etiology, severity and extent of the disease

In this paper a general presentation of the material is given The frequencies of amputations myocardial infarctions, *intracranial vascular lesions*, and the influence of age on survival rate are analysed The influence or lack of influence *on* survival and of local vascular processes in the legs or brain will be discussed

Other papers will deal with the influence of concomitant coronary heart disease (22) and the effect of anticoagulant treatment on the course of the disease (23) A preliminary report on the latter subject has been published (21)

Submitted for publication February 8 1965

Material and methods

Data were collected on 466 patients who during the period 1938—1960 had been treated at hospitals in Stockholm — mainly Sodersjukhuset Stureby vårdhem S t Erik s and S t Goran s hospitals — for complaints in the lower limbs causing a suspicion of arterial insufficiency

No upper age limit was determined One hundred patients were excluded for various reasons 31 because of incomplete data on either the patient or the disease and 69 because other diseases were more dominant than the peripheral arterial disease Patients with mitral stenosis and a history of embolic accident in the leg were excluded since the prognosis for these patients was considered to be determined mainly by the heart disease On the other hand patients were not excluded if claudication appeared in connection *with a heart infarction or if auricular fibrillation* without signs of mitral stenosis was present in spite of the possibility of embolic disease occurring in such cases.

When the peripheral arterial insufficiency appeared in the final stage of a severe disease such as heart failure generalized arteriosclerosis or cancer the patient was not included. Patients were also excluded if the circulatory

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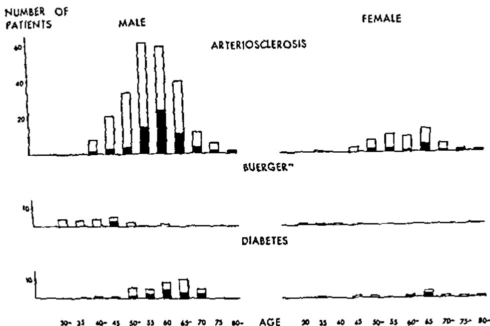


Fig 1 Age at beginning of observation period The black columns indicate patients who died during the observation period

SELECTED MATERIAL

The 366 patients selected were mainly from the Stockholm area Symptoms of arterial insufficiency in the legs had been manifest before 1958 In 212 patients a total occlusion was detected by arteriography somewhere in the area between the lower part of the aorta and the crural arteries (table II) *In* 56 patients stenosing changes of varying de gree were found but no total occlusion In these patients and in the 98 who were *not* subjected to arteriography other objective signs of obliterative arterial disease in the legs were detected by means of pulse palpa tion oscillometry etc

The observation period started when obliterative arterial disease in the lower limbs was diagnosed The patients were observed until the end of 1960 or until their death if this occurred previously

ETIOLOGY

Arteriosclerosis without diabetes

The majority of the patients were considered to have arteriosclerosis without diabetes This group arteriosclerotics comprised 294 patients The male/female ratio was 243/51

Morbus Buerger and other types of arteritis

Biopsy findings in 5 men were considered to be *typical* of Buerger's disease (3 10) In 10 men and one woman the histories and objec tive findings (relapsing thrombophlebitis arterial occlusions in the arms etc) indicated Buerger's disease Biopsy was not performed but the diagnosis Buerger's disease was con sidered to be *probable* Four men and two women exhibited no obvious signs of either arteriosclerosis or Buerger's disease and,

disturbance was regarded as due to injuries caused by cold, burns, gunshot or bullet wounds Moreover, when venous insufficiency, polyneuritis or arthrosis were considered to dominate the picture of the disease, the patients were also not included

DIAGNOSTIC PROCEDURES

The diagnosis was based on medical history, physical examination, certain laboratory investigations and arteriography, as subsequently described

History

The histories were investigated with special reference to onset symptoms and age at onset, disposing diseases and the course of the disease

Physical examination

Inspection of the colour of the skin of the lower extremities with the limbs at horisontal, elevated and hanging positions Recording of varices ulcers and other changes in the skin

Palpation of skin temperature and of pulsations in the aorta the femoral popliteal dorsalis pedis and posterior tibial arteries Absence of pulsations only in the dorsalis pedis artery was not regarded as a sign of obliterative arterial disease, for about 10 % of normal patients have no such pulsations (16) A thrill and/or a systolic murmur over an artery, indicates a stenosis situated proximally and is most commonly noted in the inguinal area

Laboratory methods

Oscillometry was applied for locating the level of a stenosis or a total occlusion using either an automatic oscillograph according to Ejrup (4) or an oscillometer of a Collen or Comparatif type As a rule the pulsations were recorded at the wrists proximally and distally on the thighs, just below the knees and just above the ankles The pulsations from the ankle region should be more prominent than those from the wrists

Skin temperature of the toes was recorded before and after vasodilatation (7, 11)

Digital plethysmography of the toes was carried out according to Lund in connection with the skin temperature recording after vasodilatation (7, 11)

The *arteriographic technique* used was described in a previous paper (21) In most patients the contrast medium was injected directly after percutaneous puncture of the femoral artery in a cranial direction The arteries were thereby visualized from the distal part of the iliac to the proximal part of the tibial and fibular arteries If obliterative changes in the aorto-iliac region were considered probable, aortography was performed either by direct puncture of the abdominal aorta or by catheter from the contralateral groin

A *walking tolerance test* according to Lund (8) was applied to ascertain the patient's functional capacity

Electrocardiography Criteria for coronary heart disease (CHD) and the classification of the patients in three groups (normal group, possible CHD and typical CHD) are described in a following paper (22)

Glucose test All the patients were subjected to a glucose urine test In cases with positive reaction fasting blood sugar was determined

THERAPEUTIC MEASURES

Most patients were treated at the out patient clinics of the different hospitals The following measures were applied in varying degree prohibition to smoke dietary prescriptions, training to walk surgical treatment treatment with vasodilators and oral anticoagulants

STATISTICS

The life table method (5) was used for studying the survival rate in different patient groups The following significance limits were used

$0.05 > p > 0.01$ for almost significant (*)
$0.01 > p > 0.001$ for significant (**)

Staffan Ekblom B A, of the Statistical Research Group University of Stockholm kindly assisted in the statistical evaluation of the material

TABLE I Onset symptoms

	Arteriosclerotics		Buerger		Diabetics	
	♂	♀	♂	♀	♂	♀
Claudication onset						
Slow	230	42	10	2	25	2
Acute	10	5	1	—	—	—
Pain at rest	—	—	1	1	1	2
Ulcer or gangrene	3	4	7	—	12	8
Total	243	51	19	3	38	12

50—69 years old, 56 (15 %) were 30—49 years and 36 (10 %) were 70 years and over The number of patients in the respective 5 year groups is given in fig 1

The mean age of arteriosclerotics was 59 (40—82) years for men and 61 (35—83) years for women of male Buerger patients 42 (30—62) years and of female 38 (32—42) years for male diabetics it was 62 (42—74) years and for female diabetics 64 (39—80) years

Duration of symptoms before observation period

At the beginning of the observation period the patients had had symptoms of arterial insufficiency in the lower limbs for on an average 3 (0—29) years (fig 2) Fifty nine patients (16 %) had exhibited symptoms for a period not exceeding one month In 21 of these patients the symptoms were manifested so suddenly that an acute arterial occlusion must be suspected One hundred and seventy one patients (47 %) had displayed symptoms for not more than one year 85 patients (23 %) for 1—3 years 41 (11 %) for 3—5 years 52 (14 %) for 5—10 years and 17 patients (5 %) for more than 10 years

The duration of the observation period was on an average 5 0 years (1—273 months) It was less than one year for 26 patients (7 %), 1—3 years for 62 patients (17 %) 3—5 years for 130 patients (35 %) 5—10 years for 123 patients (34 %) and more than 10 years for 25 patients (7 %) (fig 3)

Onset symptoms

In table I the onset symptoms are given for different categories of patients

The most common symptom was *intermittent claudication* with the pain localized in the foot calf thigh or buttock on one or both sides *At first the symptoms were often manifested* in connection with only considerable physical effort but gradually they appeared after *increasingly slighter efforts*

In some cases the arterial insufficiency revealed itself as *acute severe pains at rest* caused by a total occlusion of one of the larger leg arteries owing to a local thrombosis or an embolus

In patients with Buerger's disease or diabetes the onset symptom was often *ulceration or gangrene on the toes*

Other symptoms such as a peripheral feeling of cold or local discoloration may

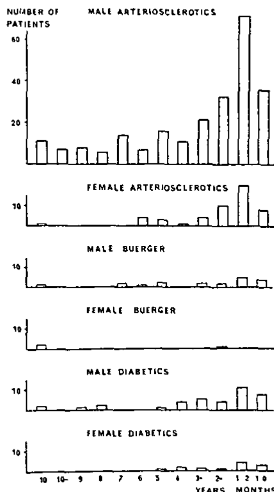


Fig 2 Duration of symptoms before observation period

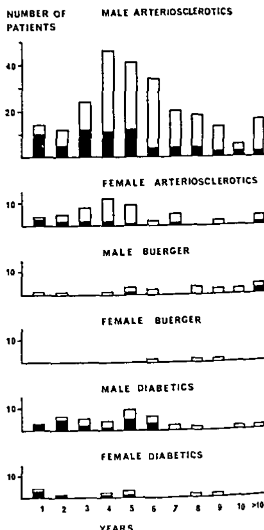


Fig 3 Duration of observation period The black columns indicate the patients who died during the observation period

consequently, their disease was designated as *unspecific arteritis*

The patients who were classified as typical or probable cases of Buerger's disease or as unspecific arteritis, were recorded as "Buerger" patients This group comprised 22 patients, none of them had diabetes The male/female ratio was 19/3 Because of the remarkable difficulty in definitely distinguishing many of these cases from arteriosclerosis the group was reported, together with those having arteriosclerosis, as being non diabetic, except as regards amputations

Arteriosclerosis with diabetes

All patients who had had glucosuria and hyperglycaemia either before or during the observation period were placed in the diabetic group No attempt was made, however, to detect cases with "latent" diabetes by means of a glucose tolerance test Already at the beginning of the observation period 41 patients were known to be suffering from diabetes The disease was subsequently diagnosed during the observation period in 9 patients

This group diabetics comprised 50 patients The male/female ratio was 38/12

Age at beginning of observation period

At the beginning of the observation period 274 (75 %) of the patients were

BLE III Final level of amputation

	Arteriosclerotics		*Buerger		Diabetics	
	♂	♀	♂	♀	♂	♀
ugh	28	3	5	—	8	2
nall of the leg	6	—	1	1	3	2
oe	2	1	4	—	13	—
otal	36	4	10	1	24	4

ABLE IV Amputated patients

	Arteriosclerotics		Buerger		Diabetics	
	♂	♀	♂	♀	♂	♀
Total number of patients	243	51	19	3	38	12
Amputated patients	32	4	9	1	19	4
Age at first amputation	64 (40-84)	67 (61-73)	40 (31-51)	20	66 (52-74)	74 (65 80)
First amputation						
Before observation period	1	1	1	1	6	1
During observation period	31	3	8	0	13	3
Observation months until amputation if any	13 834	2 438	1 104	175	1 187	427
Patients with first amputation per 1 000 observation months	2 24	1 23	7 97	—	10 95	7 02

during the same month only the last amputation was recorded In 8 cases a distal amputation was followed by a more proximal amputation after more than one month The final level of amputation is given in table III Amputations on toes only were most common among the diabetics whereas thigh amputations dominated in the arteriosclerotic group The right and the left leg were amputated with equal frequency in the arteriosclerotic group (22/18) and the Buerger group (5 6) In the diabetic group however 20 right legs but only 8 left legs were amputated, this may be due entirely to chance

Out of 294 arteriosclerotics 36 had amputations out of 22 Buerger patients 10 and out of 50 diabetics 23 (table IV) At the beginning of the observation period 11 patients had already undergone amputation and 8 of these had amputations during the observation period also Out of 355 patients who had not previously undergone amputation 58 had amputations

TABLE II Findings at first arteriography

	Non-diabetics		Diabetics	
	♂	♀	♂	♀
No arteriography	58	18	17	2
Inadequate film quality	3	–	–	–
Total occlusion				
Aortic, iliac	16	4	–	1
Superficial femoral	128	27	10	6
Popliteal	10	–	–	1
Deep femoral	1	–	–	–
Tibial, fibular	5	–	2	1
Stenosis				
Iliac	5	3	–	–
Superficial femoral	36	2	9	1
Total	262	54	38	12

be the first sign of failing arterial blood supply Buerger's disease often begins with relapsing thrombophlebitis But, for the sake of uniformity, the definite signs of arterial insufficiency, intermittent claudication or ischemic ulceration, were taken to represent the onset of the disease

Findings at the first arteriography

In 271 patients one or both of the lower limbs were investigated by arteriography The film quality was inadequate in connection with 3 of these patients and, therefore, they were regarded as not subjected to arteriography

Two hundred and twelve patients were grouped according to the most proximal total occlusion found in either limb (table II) In 56 patients no total occlusion was found and they were grouped according to the most proximal stenosis observed The superficial femoral artery was the most common site of both total occlusions and stenoses

Course of disease

Most patients were checked either once a year or more frequently The patient's own opinion on his condition was recorded and a clinical examination was made with special reference to the vascular disease This was also checked by the laboratory methods previously described In many cases femoral arteriography was carried out at intervals of about two years

The present analysis is made with no regard to the various therapeutic measures applied Other papers deal with the arteriographic changes (21), the relation between concomitant coronary heart disease and survival (22), and results of long-term anticoagulant treatment (23)

Amputations

By the end of the observation period 69 out of the 366 patients had undergone amputation, 59 on one leg only, and 10 on both legs When an amputation at the toe or ankle level was followed by an amputation at a more proximal level

TABLE V Myocardial infarctions

	Non-diabetics		Diabetics	
	♂	♀	♂	♀
Total number of patients	262	54	38	12
Patients with infarction	65	8	14	4
Age at first infarction	58 (38–72)	57 (44–78)	58 (47–75)	63 (45–73)
First infarction				
Before observation period	35	3	9	2
Before or during observation period	4	2	2	—
During observation period	26	3	3	2
Observation months until first infarction if any	13 575	2 546	1 298	477
Patients with first infarction per 1 000 observation months	1.92	1.18	2.31	4.19

TABLE VI Intracranial vascular lesions (IVL)

	Non-diabetics		Diabetics	
	♂	♀	♂	♀
Total number of patients	262	54	38	12
Patients with IVL	30	6	6	2
Age at first IVL	61 (31–83)	61 (41–73)	67 (58–72)	66 (65–67)
First IVL before observation period				
Wallenberg syndrome	1			
Homonymous hemianopsia	2			
Hemiparesis	5	1		
First IVL during observation period				
Cerebral thrombosis s. embolia	10	2	2	
Cerebral haemorrhage	1	2		1
Subdural haematoma	2			1
Hemiparesis	9	1	4	
Observation months until first IVL	15,306	2 878	1 818	486
Patients with first IVL per 1 000 observation months	1.38	1.77	3.28	4.11

during the observation period, and the frequency of amputation was calculated for these patients

The number of patients undergoing their first amputation per 1,000 observation months was 3 24 for male and 1 97 for female patients The difference is not statistically significant Owing to the small number of female patients, the frequency of amputation in the different etiologic groups was compared for men only The frequency of amputation among diabetics was almost significantly (*) higher than among arteriosclerotics The frequency was not significantly higher among Buerger" patients than among arteriosclerotics, but, as the number of Buerger" patients was small, this result has to be regarded with caution The frequency of amputation was not significantly higher for diabetics than for Buerger" patients

Myocardial infarction

By the end of the observation period 91 out of 366 patients had suffered a myocardial infarction with a typical history and/or typical electrocardiographic changes according to the criteria stated in a following paper (22) Six patients, in whom infarction changes were first detected at the post mortem examination, were not included

In 64 cases it was possible to determine the onset of the peripheral arterial disease in relation to the first heart infarction It occurred before the infarction in 45 patients, at about the same time as the infarction in 5, and subsequently in 14

At the beginning of the observation period 49 patients had already had an infarction (table V) In 5 of these a new infarction occurred during the observation period In 8 patients, whose first ECG was taken only later in the observation period and then showed signs of previous infarction, it was not possible to decide if the infarction had occurred before or after the beginning of the observation period

Out of the 34 patients who suffered from their first infarction during the observation period, 14 died in the acute stage At autopsy they all displayed signs of recent coronary thrombosis and/or fresh ischemic myocardial changes

The number of patients suffering from their first infarction per 1,000 observation months was 1 95 for all males and 1 66 for all females The frequency of infarction seemed to be higher among diabetics than among non diabetics The differences, however, were not statistically significant

Intracranial vascular lesions

Neurological symptoms such as hemiparesis, aphasia, ataxia, and hemianopsia, were interpreted as signs of intracranial vascular lesions, IVL (3a) Two patients in whom encephalomalacias were detected at autopsy but information about clinical symptoms was lacking were not included Three patients, who had had short episodes of unconsciousness with clonic spasms but without persistent paresis were excluded on account of the temporary nature of the symptoms and the uncertainty whether the cause was intracranial or cardiac Nor were 3 patients included who displayed diffuse symptoms of cerebral

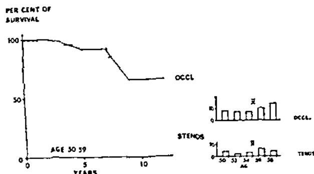


Fig 5 Percentage of survival for patients with total occlusion (—) in the femoral region compared with that of patients with stenoses only (— — —) Male non-diabetics aged 50—59 years at first arteriography Age distribution (right) Symbols as in fig 4

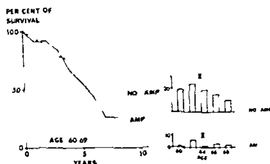


Fig 6 Percentage of survival for patients who underwent amputation (—) compared with that of patients who did not undergo amputation (— — —) Male non-diabetics aged 60—69 years at beginning of observation period Age distribution (right) Symbols as in fig 4

The survival curves were calculated according to the life table method (5) For the male population of Stockholm at the ages of 45 55 and 65 years the survival curves were based on the data given in the Statistical Abstract of Sweden 1959 (dotted lines in fig 4) These curves are computed for such a large material that the 95 per cent confidence intervals coincide with the dotted lines

The survival curves for patients were calculated for male non-diabetics aged 40 49 50 59 and 60—69 years The mean ages $\bar{x}$ were 45 4 55 4 and 64 0 years respectively The age distribution is given on the right of fig 4 These three groups consisted of 38 97 and 100 patients respectively 8 19 and 36 of whom died during the observation period, the average period of survival being 83 (2—273) 73 (1—187) and 49 (2—143) months respectively The curves for the patients were almost significantly (*) lower than those for the average population 16—17 years 10—13 years and 4—9 years after the beginning of the observation period in the respective age groups

Survival of patients with occlusions in the femoral region compared with that of patients with stenoses only

Among the male non-diabetics who were 50—59 years old at the beginning of the observation period 46 patients

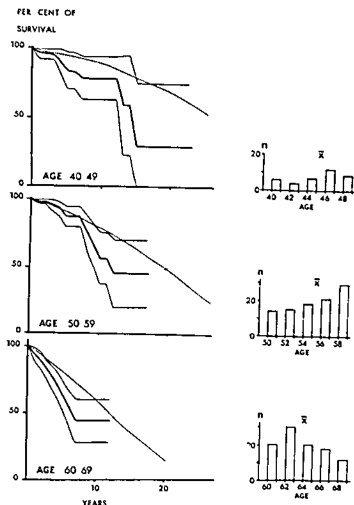


Fig 4 Percentage of survival at different periods after beginning of observation period for male non-diabetic patients aged 40—49, 50—59 and 60—69 years (—) and general male population aged 45, 55 and 65 years ( ) The latter curves lie partly outside of the 95 % confidence intervals (—) to the respective patient curves thus indicating lower survival rate for patients than for mean population (Right) Age distribution at beginning of observation period (n = no of patients x̄ = mean age)

sclerosis, such as deterioration of memory, dizziness or mental confusion

By the end of the observation period 44 of the 366 patients had had signs of IVL (table VI) Nine patients already had had an IVL before the beginning of the observation period, and two of these also had one or more lesions during the observation period

Out of the 357 patients who had had no IVL previously, 35 had one during the observation period, and 18 of the latter patients died in connection with the lesion The number of patients, per 1,000 observation months, who had their first IVL was 1 58 for all males and 2 11 for all females These figures appeared to be higher for diabetics than for non diabetics The differences, however, were not statistically significant

Survival rates

During the observation period 109 out of the 366 patients died The total mortality in the different age groups is shown in fig 1 In the arteriosclerotic group 68/243 males and 12/51 females died, in the Buerger group 3/19 males and 0/3 females and in the diabetic group 19/38 males and 7/12 females The length of the observation period until death occurred is given in fig 3

TABLE VII Causes of death

	Non-diabetics		Diabetics	
	♂	♀	♂	♀
Total number of patients	262	54	38	12
Deaths	71	12	19	7
Causes of death				
Arteriosclerotic heart disease				
Acute	11	3	2	1
Chronic	4	1	3	—
Myocardial infarction	12	—	4	2
Cerebral thrombosis	9	2	2	—
Other vascular diseases	10	3	4	2
Haemorrhages	5	2	2	2
Infections postoperative deaths etc	8	1	2	—
Cancer	12	—	—	—

Causes of death

Causes of death were obtained from the death certificates and in the majority of cases these were based on autopsy In 76 out of the 109 patients who died during the observation period the cause of death was considered to be different manifestations of the occlusive arteriosclerotic disease 11 patients died of haemorrhage 12 of cancer and 10 of various causes including infections post operative deaths etc (table VII)

Acute arteriosclerotic heart disease was defined as acute cardiac death without signs of coronary thrombosis and/or recent myocardial ischemic changes This cause of death together with acute myocardial infarction was dominant in both the non diabetic and the diabetic groups 26 83 and 9 26 respectively Cancer occurred only in male non diabetics and pulmonary carcinoma was most common 8 cases)

In 2 patients who showed both recent softening of the brain and subdural haemorrhage the latter was recorded as the cause of death One woman with a fatal hemiplegia was registered as a case of cerebral haemorrhage, although no autopsy was performed and her history rather indicated a cerebral thrombosis A pulmonary embolus was stated to be the cause of death of one patient, but he had been treated for lung cancer some months before his death and this disease was given as the cause of death in the present report

In a following paper (23) the causes of death will be considered in relation to anticoagulant treatment

Discussion

The purpose of the investigation was to obtain a basis for the evaluation of the effects of long term anticoagulant treatment on patients with obliterative arterial disease in the lower limbs

The material consisted of 300 men and 66 women who had had symptoms of arterial insufficiency of the lower limbs

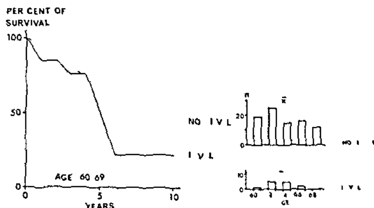


Fig 7 Percentage of survival for patients who had intracranial vascular lesion (IVL) (—) compared with that of patients who were not injured (— — —) Male non-diabetics aged 60—69 years at beginning of observation period Age distribution (right) Symbols as in fig 4

had a total occlusion in the femoral region at the beginning of the observation period, whereas 19 patients had stenoses only The mean age ($\bar{x}$) at the beginning of the observation period was 55 2 and 55 0 years, respectively Age distribution is shown on the right of fig 5 The duration of the observation period was 70 (11—187) months and 76 (20—140) months, respectively

During the first 6—7 years the survival curves followed the same course, but thereafter the stenoses curve was steeper than the occlusion curve The difference was not statistically significant at any point of the curves

Survival of patients with and without amputations

Among the male non diabetics, who were 60—69 years old at the beginning of the observation period, 13 patients underwent amputation during the observation period and 87 patients did not The mean age ($\bar{x}$) at the beginning of the observation period was 64 2 and 63 9 years respectively The age distribution is shown on the right of fig 6 The duration of the observation period was 49 (2—143) and 55 (8—90) months in the respective groups

The survival curve for the patients who were amputated had a slightly steeper course than that for the non amputated patients, but this difference between the curves was not statistically significant

Survival of patients with and without intracranial vascular lesions (IVL)

Among the male non diabetics who were 60—69 years old at the beginning of the observation period, 13 patients had an intracranial vascular lesion (IVL) during the observation period and 87 patients had none The mean age ($\bar{x}$) at the beginning of the observation period was 63 8 and 64 0 years respectively The age distribution is shown on the right of fig 7 The observation period was 50 (8—143) and 49 (2—106) months, respectively

The survival curve for the patients who developed IVL had a slightly steeper course than that for the patients with no IVL but this difference between the curves was not statistically significant

in the present material was larger than in the average population The survival curves were calculated from the beginning of the observation period and not from the onset of symptoms, since in many cases the time of onset was uncertain, furthermore, it was impossible to ascertain how many patients had died before the observation period '

The difference in the corresponding age groups between the curves for the patients and those for the general population was almost significant (*) for part of the curves The difference was most pronounced in the age group 60—69 years

Patients with total occlusions in the femoral region had the same survival rate as those with stenoses only at least in the age group 50—59 years during the first seven years Patients who underwent an amputation before and/or during the observation period had a slightly lower (not significant) life expectancy than patients not undergoing amputation These findings may indicate that the course of the disease in the lower limbs does not affect life expectancy to any considerable extent

Patients who suffered an intracranial vascular lesion during the observation period seemed to have a slightly lower (not significant) life expectancy in comparison with that of patients without such a complication

Coronary heart disease is the most common sign of a generalization of the obliterative arterial disease and its influence on survival will be dealt with in a separate paper (22)

As could be expected in most cases the recorded causes of death were connected with the generalized arteriosclerosis This basic disease must be regarded as a contributory cause of death also in cases, where other conditions, such as haemorrhage, infections, cancer, were recorded as the main cause of death In most patients the cause of death was determined on autopsy However, to state which is the principal cause of death among numerous contributory conditions must be regarded as rather arbitrary in many cases, e g when recent myocardial infarction, brain softening and subdural haemorrhage were found in the same patient on autopsy

Summary

From four hospitals in Stockholm 366 patients were collected (male female ratio 300,66) who had had symptoms of obliterative arterial disease in the lower limbs before 1958 The course of the disease as regards survival rate amputations myocardial infarctions and intracranial vascular lesions was studied up to the end of 1960 or until death of the patient if this occurred before this date Arteriosclerosis without diabetes was present in 294 patients Buerger's disease and other types of arteritis in 22 and arteriosclerosis with diabetes in 50

On an average the patients had had symptoms of arterial insufficiency for 3 years before the observation period This period in turn averaged 5 years

The patients with Buerger's disease were reported separately only in respect

before 1958 This male/female ratio does not reflect the morbidity In the Basel chemical industries, Widmer (24) found that for workers in the age group 40—64 years this condition was only 1 6 times more frequent in men than in women

Arteriosclerosis without diabetes was diagnosed in 294 patients, Buerger's disease and other types of arteritis in 22, and arteriosclerosis with diabetes in 50 The number of patients with Buerger's disease and other types of arteritis was larger than would be expected It was often difficult to distinguish between Buerger's disease and arteriosclerosis, especially in older patients Therefore, these two categories of patients were reported together as 'non diabetics", except in the analysis of amputations In most diabetics the presence of this disease was already known at the start of the observation period No attempt was made to find latent diabetes by means of a glucose tolerance test

On an average, the patients had displayed symptoms of obliterative arterial disease in the lower limbs for three years *before the beginning of the observation* period In most cases the symptoms developed slowly Thirty per cent of the patients stated that they had had symptoms for more than three years when they first consulted a doctor on this account This indicates the benign course that the disease may run for several years in many patients

The first arteriography of the lower limbs disclosed total occlusion of the superficial femoral artery in 64 per cent Development of total occlusions and appositional growth of existing total occlusions were reported on in a previous paper (21) and will be discussed more fully in a later publication (23)

Patients who had suffered one amputation were more prone to require further amputations than were patients without any such previous complication The same applied to intracranial vascular lesions Patients with a myocardial infarction have a 5—10 times greater risk of a new infarction than the general population (14a) Therefore, only the first complication of each kind was reported

The frequency of the respective complications was calculated per 1,000 observation months Hence, patients were excluded who had suffered from a complication before the observation period

The Buerger" patients were reported on separately only with regard to amputations The amputation frequency was higher in the Buerger" patients than in the arteriosclerotics, but lower than in the diabetics The differences, however, were not significant Male diabetics had an almost significantly *higher amputation frequency than had* arteriosclerotics without diabetes Also as regards myocardial infarctions and intracranial vascular lesions the diabetics showed higher frequencies, but the differences were not significant, as the diabetic group was small

The survival curves were calculated for male non diabetics aged 40—49, 50—59 and 60—69 years at the beginning of the observation period Patients with diabetes were excluded, as this disease in itself involves an increased death risk, and the number of diabetics

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to amputations. Their frequency of amputation seemed to be higher than that for non-diabetic arteriosclerotics, but lower than that for diabetics. The diabetics had a higher amputation frequency than the arteriosclerotics. The former seemed also to have a higher frequency of myocardial infarctions and intracranial vascular lesions than the non diabetics.

During the observation period 109 patients (30 %) died.

Survival curves for non diabetic males 40—49, 50—59 and 60—69 years respectively, indicated that survival for these patients was almost significantly shorter than for the general population in the corresponding age groups.

No significant difference in survival existed between patients with total occlusion in the femoral region and patients who had only stenoses. Nor was there any obvious difference in survival between patients with and without amputations.

The role of concomitant coronary heart disease will be dealt with in a separate paper (22).

As regards causes of death, those dominated which were connected with the generalized arterial disease (70 %). In very few patients was the obliterative arterial disease of the lower limbs regarded to be the principal cause of death.

When evaluating medical and surgical therapy in obliterative arterial disease of the lower limbs, factors such as etiology, age, concomitant coronary heart disease and cerebrovascular disease should be taken into consideration.

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Obliterative Arterial Disease of the Lower Limbs

III *Prognostic* Influence of Concomitant Coronary Heart Disease

By

Carl Thulgren

Most patients who consult a doctor because of obliterative arterial disease in the lower limbs do not die of this disease, but rather of cardiac or cerebral complications due to generalized vascular disease Objective signs of such a generalization e g in the electrocardiogram may thus indicate a worse prognosis for the patient Contradictory information on the prognosis of patients with obliterative arterial disease in the legs can probably be largely explained by the varying development of the cardiac and cerebral manifestations in different patient materials

The purpose of the present study is to illustrate the prognostic significance of concomitant coronary heart disease (CHD) in patients with obliterative arterial disease in the lower limbs

Material

In all 366 patients in whom symptoms of arterial insufficiency in the legs occurred before 1958 were followed up until the end of 1960 or until death if this took place previously The observation period started when the obliterative arterial disease in the lower limbs was diagnosed and it varied between 1 *month and 23 years More details* on the material were reported in a previous paper (13)

The investigation was retrospective with regard to a considerable part of the material *The histories of heart trouble were incom*plete and in many cases information was *entirely lacking On the other hand* most patients had undergone ECG examination Consequently the grouping of the material *in respect of cardiac condition* was mainly based on the ECG findings

For 20 patients ECG was not recorded For another 18 patients the ECG curves were not available at the final examination *The ECGs of the remaining 328* patients could be checked At the beginning of the observation period ECG data on 274 patients were available which made classification possible according to the following criteria

If the ECG was normal at the beginning of the observation period but had previously shown signs of typical or possible coronary heart disease (CHD) the patient was placed in the respective CHD group

In 54 patients, the first ECG was taken more than 3 months after the beginning of

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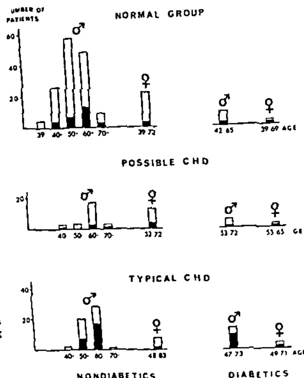


Fig 1 Patients grouped according to cardiac findings at the beginning of the observation period Only male non-diabetics are divided into age groups The black columns indicate patients who died during the observation period

tion period Out of these 328 patients 178 were assigned to the normal group 53 to the possible CHD group and 97 to the typical CHD group

In the normal group 24 patients developed signs of typical or possible CHD during the observation period This is a minimum figure because with regard to 100 patients in this group a repeated ECG was not recorded during the last observation year Thus at the end of the observation period 174 (53 %) of the 328 patients had displayed signs of possible or typical CHD

Among the 53 patients with possible CHD 36 had more or less pronounced signs of left ventricular strain Of these patients 31 had varying degrees of hypertension Only one patient had been diagnosed as suffering from stenosis of the aortic valve

Mortality

The 26 patients whose first ECG examination was made later during the observation period and had then exhibited signs of possible or typical CHD were excluded from the following analysis The distribution of the remaining 302 patients according to etiology and sex is shown in fig 1 Only male non diabetics were divided into age groups

In the normal group 34 out of 178 patients died during the observation period The corresponding figures were 10 out of 47 patients in the group with

the observation period The 28 patients, whose ECG was then normal, were included in the corresponding age group at that time On the other hand, the 26 patients who exhibited signs of typical or possible CHD, were not included in the survival analysis, since clinical deterioration might have led to an investigation with ECG, and thus could have distorted the results

Method

During the observation period, which for some patients began at the end of the 1930s, the ECG technique varied at the different Stockholm hospitals Leads I—III together with one or two chest leads represented the routine procedure in the 1940s during the 1950s an increasing number of patients were investigated with 11—12 leads, including unipolar limb leads and several precordial leads

Originally the ECGs were examined by different physicians In order to obtain a uniform and impartial assessment of the material a cardiologist, Dr Viktor Kohler (5), otherwise not engaged in the study, examined all the available ECGs Attention was paid to possible digitalis therapy and to the following criteria of CHD which were suggested by Biorck (1) and Blomqvist and Astrand (2)

NORMAL GROUP

1 *Normal ECG* at beginning of or during observation period and no history of myocardial infarction
2 *Incomplete right bundle branch block* i e QRS < 0 12 sec

POSSIBLE CORONARY HEART DISEASE

1 *Possible coronary insufficiency*
a Slight depression of S T junction (not exceeding 0 5 mm) *but* S T horizontal or descending
b Isolated depression of S T junction of 1 0 mm or more and S T ascending
2 *Left ventricular strain* Diphasic T in left ventricular leads especially V_6 and V_7 as well as S T depressions As a rule, the negative phase of the T waves is dominant, but usually with a positive final phase (3) R waves large or normal in the left ventricular leads When the electrical axis is in a median position, T_{II} and T_{III} can be negative with depressed S T

In the cases where only 4 or 5 leads were taken (I, II, III, CR_4 and possibly CR_1), it may be impossible to decide whether coronary insufficiency or left ventricular strain is present Even if all 11—12 leads are used it may be difficult to distinguish signs of coronary insufficiency from left ventricular strain
3 *Auricular fibrillation*, transient or constant

TYPICAL CORONARY HEART DISEASE

1 *Typical history of acute infarction before observation period* Either the ECG in the acute phase showed the course of infarction with the development of changes in the QRS and S T-T, or later the ECG displayed signs of previous infarction with a pathological Q or QS complex, abnormal R progression or deeply inverted T waves
2 *No typical history of acute infarction* but ECG before or at the beginning of the observation period showed signs of passed infarction
3 *Typical coronary insufficiency* Depression of S T junction of at least 0 5 mm *and* S T horizontal or descending

In the results no subdivision is made within the main groups normal possible CHD, and typical CHD

Results

In the majority of cases the previous interpretations of the ECGs were verified and minor corrections were made with regard to only 36 patients

It was possible to classify 274 patients on the basis of their ECG at the beginning of the observation period, a further 54 patients had their first ECG recorded only later during the observa

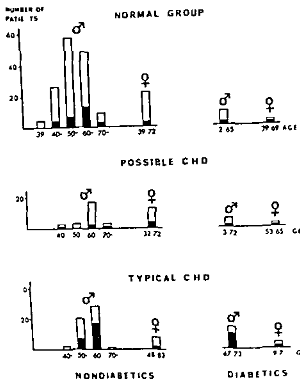


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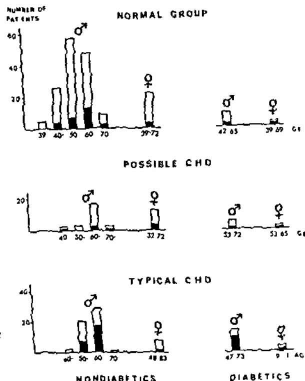


Fig. 1. Patients grouped according to cardiac findings at the beginning of the observation period. Only male non-diabetics are divided into age groups. The black columns indicate patients who died during the observation period.

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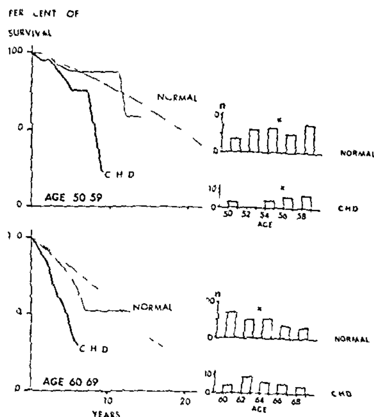


Fig 2 Percentage of survival at different periods after beginning of the observation period for male non-diabetic patients aged 50—59 and 60—69 years. — = groups with typical CHD, — = normal groups ---- = general male population aged 55 and 65 years respectively (Right) Age distribution at beginning of observation period (n = no. of pat., $\bar{x}$ = mean age)

possible CHD and 38 out of 77 patients in the group with typical CHD The mortality, indicated by black columns in fig *1*, was considerably higher in the group with typical CHD than in the normal group On the other hand, the group with possible CHD did not seem to differ from the normal group in this respect

Survival rates

The presence of typical CHD apparently signifies an increased risk of death A more reliable measure of this was obtained by calculating survival curves according to the life table method (4) This was feasable (fig 2) for the normal groups (thin continuous lines) and the groups with typical CHD (heavy continuous lines) among male non diabetics aged 50—59 and 60—69 years The other groups of patients were too small for this method to be applied The survival curves for the male population of Stockholm at the ages 55 and 65 years (dotted lines) were based on the data given in Statistical Abstract of Sweden 1959 (*11*)

The *normal groups* comprised 57 and 48 patients respectively and their mean ages at the beginning of the observation period were 55 1 and 63 6 years respectively The duration of the observation period was 68 (16—161) and 53 (7—143) months In general the survival curves seemed to correspond to those for the same age groups in the mean population at least during the first 10 observation years

Table I Arteriographic findings at beginning of observation period

	Male non-diabetics					
	Normal group			Group with typical CHD		
		Arteriography group			Arteriography group	
Age	Total	I II	III	Total	I II	III
50 59	47	11	36	15	5	10
60 69	38	11	27	17	1	16

The *groups with typical CHD* comprised 20 and 28 patients respectively whose mean age at the beginning of the observation period was 55 9 and 64 3 years respectively The duration of the observation period was 60 (1—104) and 46 (2 90) months respectively The course of the survival curves was considerably steeper than that for the normal groups and the mean population

In the age group 60—69 years, a life expectancy of 5 years was significantly (**) lower for patients with typical CHD 0 402 ± 0 100) than for the patients in the normal group (0 769 ± 0 070) A life expectancy of 6 years was also significantly (**) lower in the CHD group 0 287 + 0 099) than in the normal group (0 666 ± 0 091) With regard to a life expectancy of 1—4 years and more than 6 years the material was too small for statistical analysis and so was the group with typical CHD aged 50 59 years

Relation between presence of CHD and arise of peripheral arterial disease

This investigation included only male non diabetics who were 50—69 years of age at the beginning of the observation period and who belonged to the normal group or the group with typical CHD

Arteriographic findings in the femoral popliteal and deep femoral arteries were grouped according to statements in a previous paper (12) as follows

Group I mild stenoses without visible collateral vessels

Group II considerable stenoses with visible collateral vessels

Group III total occlusion

If both legs were subjected to arteriography the patient was classified according to the most severe lesions found

Arteriographic propagation was defined as the development of total occlusion in a vessel that was only stenosed previously (groups I and II → group III) and also as the appositional growth of an existing or the development of a new total occlusion in the same vascular region

Propagation tendency was defined as the number of patients showing propagation per 1 000 observation months

Initial arteriographic findings

In all 117 patients underwent arteriography 85 in the normal group and 32 in the CHD group (table I) In the age group 60—69 years there were more patients with total occlusion in the CHD group than in the normal group but

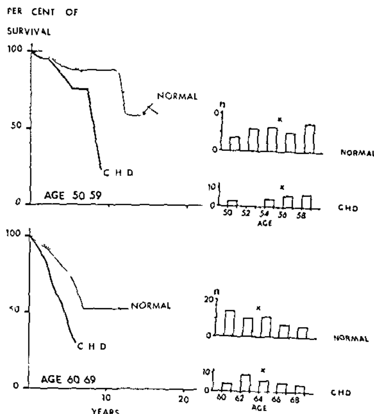


Fig 2 Percentage of survival at different periods after beginning of the observation period for male non diabetic patients aged 50—59 and 60—69 years — — groups with typical CHD, — — normal groups = general male population aged 55 and 65 years respectively (Right) Age distribution at beginning of observation period (n = no of pat x̄ = mean age)

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with or after the symptoms from the lower limbs The longer there is a possibility to follow up the patients with this disease, the more usual become the signs of CHD Gradually, some patients suffer more from CHD than from obliterative arterial disease of the lower limb However, the reverse condition is also encountered *Patients with* angina pectoris can become free from heart trouble when intermittent claudication is manifested since their leg complaint prevents them from moving rapidly

In the present material, which was partly studied retrospectively, the CHD diagnosis was mainly based on ECG findings The frequency of CHD observed has to be regarded as a minimum value If the present ECG technique with 11—12 leads had been applied in all cases completed with an ECG after exercise in patients with normal or suspect ECG the frequency of typical CHD changes would certainly have been higher

At the beginning of the observation period the diabetics showed a higher frequency of typical CHD than did the non diabetics This is partly due to the fact that the diabetics were older than the non diabetics

The statistical analysis was confined to male non diabetics aged 50—69 years *In the arteriographic follow* up the development of total occlusions as well as appositional growth of existing total occlusions was about the same both in the typical CHD group and in the normal group The frequency of amputations was also the same in both groups Thus the occurrence of CHD *does not* appear to affect the peripheral manifestations of the vascular disease in an unfavourable direction

In the age group 60—69 years patients with typical CHD had a significantly lower life expectancy than had patients with normal cardiac findings A similar tendency was observed in other age groups but these were too small *for statistical analysis*

The occurrence of cardiac death in the typical CHD group was almost significantly higher than in the normal group aged 60—69 years This tendency however, was not observed in the age group 50—59 years

Summary

Among 366 patients with obliterative arterial disease in the lower limbs the cardiac condition was defined for 328 mainly by electrocardiography The material was divided into three groups normal possible coronary heart disease (CHD) and typical CHD

CHD possible or typical was present in more than half of the material by the end of the observation period

The frequency of amputation was not higher in the typical CHD group than in the normal group Nor did an arteriographic follow up show any difference between the two groups regarding the development of total occlusions and appositional growth of total occlusions

Patients without signs of CHD had about the same life expectancy as that of the mean population in the same age group but patients with signs of typical CHD had a lower life expectancy

the difference is not statistically significant

Arteriographic propagation

Films from 38 patients could be compared with regard to arteriographic propagation thus defined

In the *normal group* 14 out of 30 patients showed propagation In the age group 50—59 years the frequency was 11/21, and in the group 60—69 years it was 3/9 The duration of the observation period in the respective age groups was 45 (15—124, total 946) and 32 (4—131, total 387) months The propagation tendency was 11 6 and 7 76, respectively

In the group with *typical CHD*, 5 out of 8 patients showed propagation In the age group 50—59 years the frequency was 3/6, and in the group 60—69 years 2/2 The duration of the observation period was 43 (5—84, total 259) and 36 (33—39, total 72) months respectively The propagation tendency was 11 6 and 27 8, respectively

There was no difference between the normal group and the group with typical CHD aged 50—59 but some difference in the age group 60—69 years as regards arteriographic propagation tendency The material was too small to permit any relevant comparisons being made

Frequency of amputation

Out of 153 male non diabetics in the age group 50—69 years, 19 underwent amputations during the observation period none of these before the observation period

In the normal group 14 out of 105 patients underwent amputations In the age group 50—59 years the frequency was 7/57, and in the group 60—69 years 7/48 The duration of the observation period for the respective age groups was 61 (2—161, total 3,482) and 49 (4—143, total 2,342) months The number of patients per 1,000 observation months who underwent amputation was 2 01 and 2 98, respectively

In the typical CHD group 5 out of 48 patients underwent amputation with frequencies of 2/20 and 3/28 for the above-mentioned age groups The duration of the observation period was 56 (1—104, total 1,114) and 44 (2—90, total 1,236) months respectively The frequency of amputation was 1 79 and 2 42 per 1,000 observation months respectively, and thus it seemed to be somewhat lower than in the normal groups The differences, however, were not significant

Causes of death

In male non diabetics aged 50—59 years cardiac deaths occurred in 3 out of 7 patients in both the normal group and the group with typical CHD In the age group 60—69 years, on the other hand, cardiac deaths occurred in 2 out of 13 patients in the normal group and in 9 out of 17 patients in the CHD group and this difference is almost significant (*)

Discussion

Coronary heart disease (CHD) is common in patients with obliterative arterial disease of the lower limbs Symptoms of CHD can occur before, concurrently

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From The Red Cross Hospital The Hague The Netherlands

The Cause of Bleeding During Anticoagulant Treatment

By

J Roos and H E van Joost

The occurrence of bleeding during treatment with indirect acting anticoagulants seems to be an inevitable consequence A causal relationship is generally accepted The purpose of this paper is to warn against attributing haemorrhages under anticoagulants to these drugs only

In medical literature the frequency of coumarin bleeding is indicated as being between 2 ½ % and 27 % of the patients treated 1 2 3 4) The explanation of this wide variation is the incomparability of the material of several authors 1 what is called a haemorrhage? 2 what kind of therapeutical level is pursued? 3 how is the material composed as to age diagnosis accidental disease or drugs cooperation of the patients as well as duration of treatment? 4 what method of laboratory control is used? Sevitt and Innes 5) found no proof that the number of haemorrhages appearing on the same therapeutic level was dependent on whether the Quick test or the thrombotest was used

The The Hague Thrombosis Service (founder Dr H Boom 1952) gives us the opportunity to examine a number of patients treated uniformly They are referred to our Service either by the general practitioner or the consultant Our method of control is the thrombotest, we aim at 110 seconds 11 %) The patient s blood is withdrawn at home or in the out patients clinic by specially trained nurses After having performed the thrombotest the nurse communicates the dosage of the next period (varying from one day to three weeks) to the patients by postcard or telephone

The drugs most frequently prescribed are dicoumarol dicumol) phenprocoumon marcoumar) or acenocoumarol sintrom

In 1963 8 691 cases were controlled during 60 002 months of treatment 5 731 males and 2 960 females Of this total 310 = 3 6 % showed a bleeding episode which means 1 bleeding in 16 treatment years There were 2 lethal haemorrhages cerebral bleedings)

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cerebral, nasal and ocular haemorrhages (17 cases) Among the patients with bleeding from a pathological lesion 70 % (98 cases) showed a thrombotest below 140 seconds Unfortunately, we are not able to give data on the occurrence of these local lesions in patients not showing a bleeding episode in many cases the haemorrhage was the first sign of the local disease

Among the 220 bleedings 72 (33 %) were found to have occurred soon after taking another drug In 1963 some 116 other disregulations (without bleeding) were found after prescription of another drug All kinds of drugs were present, especially antibiotics diuretics hormones phenylbutazone salicylates and incidentally vitamin B It should be kept in mind however that these disregulations were also observed without other drugs while these drugs were also prescribed without disregulations A causal relationship between the aforementioned drugs and the disregulation of anticoagulant treatment therefore could not be proven

Summary

The frequency of bleeding during anticoagulant treatment in a Thrombosis Service was calculated at one bleeding during 16 treatment years (aim Thrombotest 110 seconds) Among 220 bleeding episodes, 141 (64 %) were diagnosed occurring together with local disease of the bleeding organ

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1 NAEGELI TH MATIS P GROSS R RUNGE H & SACHS H W Die thromboembolischen Erkrankungen 2nd Ed Thieme Verlag Stuttgart 1960
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TABLE I Bleeding in relation to thrombotest

220 bleeding episodes	%
Thrombotest	
> 140 sec	26
< 140 sec > 100 sec	46
< 100 sec	24
Unknown	4

TABLE II 220 bleedings in relation to organ

Bleeding organ	No	%	Local disease (%)
Brain	7	3	100
Eye	29	13	69
Nose	39	18	87
Mouth	5	2	0
Lung	4	2	100
Kidney	86	40	43
Stomach	16	7	62
Intestine	24	11	79
Vagina	10	4	50

TABLE III Bleeding in relation to local diseases or drugs

220 bleeding episodes		%
Local disease	both 10 %	64
Recently prescribed drug		33
No cause		13

In the group of recurrences, above 48 relapses we also took into account 224 patients who died, although death will not always be caused by a relapse The indication for anticoagulant treatment was venous disease in 37 %, out of the 63 % of arterial cases, 46 % suffered from coronary disease

We had the opportunity to analyse a number of 220 haemorrhages, of the remaining 90 we were not able to obtain sufficient information We defined a haemorrhage as a bleeding episode for which the patient requested medical assistance Of the 220 haemorrhages, 112 patients were older than 65 years, these 112 form 3 8 % of all patients over 65 years, whereas 1 9 % of the patients under this age had a haemorrhage This difference is statistically significant ($p < 0\ 0001$) One hundred and thirty-one bleeding episodes occurred in male and 89 in female patients This might indicate that women showed a relatively higher bleeding risk (3 %) than men (2 3 %) but on further statistical analysis the age and sex factors seem to interact the effect of age on the bleeding frequency is significant in female ($p < 0\ 01$), but not in male ($p > 0\ 10$) patients

The 220 bleeding episodes were classified according to the outcome of the thrombotest on the day of bleeding (table I) Only 26 % appeared to occur while having a thrombotest of over 140 seconds (7 2 % coagulation activity), whereas 70 % were seen in or below the so called therapeutic zone

These data became more comprehensible after a search for local disease in the bleeding organ Such a local abnormality was detected in 64 % of the haemorrhages Among them we found ulcers, cancers, haemorrhoids, inflammations and stones Table II indicates the distribution of the bleeding according to organ Cerebral nasal pulmonary and gastrointestinal haemorrhages showed the highest percentage of local lesions Hypertension was frequent in

cerebral, nasal and ocular haemorrhages (47 cases) Among the patients with bleeding from a pathological lesion, 70 % (98 cases) showed a thrombotest below 140 seconds Unfortunately, we are not able to give data on the occurrence of these local lesions in patients not showing a bleeding episode in many cases the haemorrhage was the first sign of the local disease

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From the Department of Internal Medicine (Head H Lagerlof, M D) and King Gustaf V s Research Institute (Head G Birke M D) Karolinska Hospital, Stockholm, Sweden

Errors and Artefacts in Serum Folic-acid Assays

Effects of Age, Food, Drugs and Radiation

By

Peter Reizenstein

Since 1959 (1) it has been possible to assay the human serum folic acid activity (SFAA) in various clinical conditions Low values have been found as expected, in patients with intestinal malabsorption and malnutrition (10 14), in megaloblastic anemia of pregnancy (10, 23) and in experimentally produced folic acid deficiency (12) It was unexpected however to find low values in leukemia and cancer (13, 18) and in rheumatoid arthritis (5 7) This apparent non specificity of low SFAA made it necessary to ascertain whether the SFAA is influenced by factors such as food, drugs seasonal variations, and the possible presence of growth inhibitors in the serum Errors of the method were also studied

Material

Forty six healthy volunteer controls many of them blood donors between 20 and 59 years of age were studied From 16 of them blood samples were drawn about 1—2 hours after breakfast the rest had been fasting overnight To study possible seasonal variations samples were taken during all seasons of 1963

Method

All glassware was soaked in chromic acid overnight washed thoroughly with tap water and rinsed in glass-distilled water Glass-distilled water was used for all media buffers and standard folic acid (FA) solutions

Preparation of serum samples

10 ml blood was collected in centrifuge tubes containing 1 mg of dry ascorbic acid The blood was allowed to clot and serum samples were diluted with 5×10^{-2} M sodium phosphate buffer (pH 6 1) in which ascorbic acid had been freshly dissolved to give 50 mg % If stored the serum was frozen

The serum buffer solution was autoclaved for 2 minutes at 118 C (1 kpm/cm²) Coagulated proteins were centrifuged off and the clear supernatant was diluted 1 2 5 with distilled water Portions of diluted serum 0 5 ml 1 0 ml and 2 0 ml were added in duplicate to 2 0 ml of the double strength basal medium Distilled water was added to a final volume of 4 0 ml Tubes were plugged with cotton sterilized by autoclaving at 118 C for 2 minutes cooled

Submitted for publication January 15 1965

TABLE II Age a at ons in normal adult human SFAA values

Age group	No of subjects	Mean ± S E of mean
20 30	7	4 38±0 36
31 40	10	3 98±0 47
41 50	10	4 37±0 45
51 60	7	4 91±0 91
61 90	11	4 85±0 51

ed noculated and ncubated at 37 C in a wa er bath for 20 hours The growth was n easured n a Beckman B Spectrophotometer at 660 mμ The un noculated blank control was set at 100 % transm ttance and the relat e transm ttance of other tubes deter m ned

Transm ttance as then plotted against the logar thm of the FA con entra on (Fig 1) FA act es of serum samples were calculated us ng the standard curve

Effects of rad othe apy were stud ed n 12 pa ents w h mal gnant tumours prev ously described (18) n whom weekly SFAA assays we e pe fo med dur ng 3—6 weeks of rad otherapy Da ly percen age SFAA decreases dur ng this per od were calcula ed as 1 log e x reg ess ons of log SFAA on t ne u ng an IBM 650 computer

Results

S rum pro e ns

The er or of the method 2– ²x S D of d ffere ce betw een dupl cate assays) un der the p e e t exp erimental cond tions as 14 6 %

As t all b oassays standard curves d ffered f om assay to assay but paral lell m and reproduc b l ty are con dered acceptable f 1)

Co ce abl serum prote s could comb ne w th fol c ac d mak ng t n

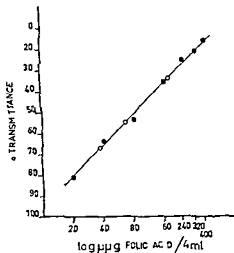


Fig 2 S mular y f L case response to normal human serum (O) and that to solu on of crystal l ne folic ac d (●) does not suggest non spec fic s mula on of L casei grow h by serum

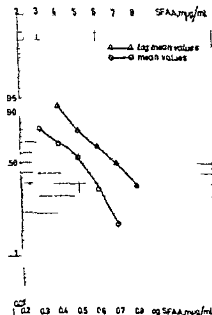


Fig 3 Norm al lis bu n plot of means of g oups of SFAA alues f o n normal con rols The loga th ms f the alues are closer o a nor mal d s r bu on

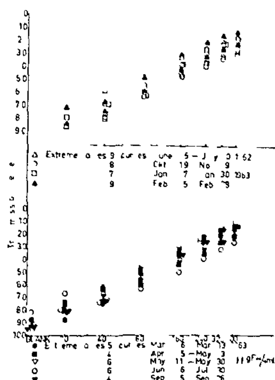


Fig 1 Highest and lowest transmission values from 9 groups of standard FA curves (a total of 58 assays), showing acceptable reproducibility of standard assay

and inoculated along with the standard folic acid assay tubes

Maintenance of assay organism and preparation of inocula

L casei (ATCC 7469) was maintained in a yeast extract protease peptone medium (1) and stored at 4° C Every 1 week 1 drop of the stored liquid culture was transferred to 10 ml of the fresh maintenance medium, incubated 18 hours at 37° C in a water bath and then stored at 4° C On the day prior to the assay one drop of an 18 hour culture was transferred into 4 0 ml of single strength basal medium (21), supplemented with 400 $\mu\mu$g FA, and incubation was performed at 37° C for 6 hours This 6 hour culture was centrifuged and washed twice with 5 0 ml of freshly prepared sterile saline (0 9 %) It was then resuspended in 5 0 ml of saline and 1 0 ml of the suspension diluted 1 25 with saline The assay tubes were inoculated with one drop of this suspension The basal assay medium has been described (21)

Standard folic-acid solution

A concentrated stock FA solution (0 5 mg/ml) was prepared in 10 $^{-2}$M NaOH, stored frozen in an amber bottle, and diluted freshly for each assay, to contain 200 $\mu\mu$g FA/ml

Assay procedure

A standard FA curve was prepared for every assay 2 0 ml double strength basal medium was dispensed into clean dry test tubes From 0 2 ml to 2 0 ml of the standard FA solution was added in triplicate to provide 20 40 60 160, 240 320 and 400 $\mu\mu$g FA The final volume was brought to 4 0 ml with distilled water Blanks with no added FA were included The tubes were plugged with cotton and autoclaved for 2 minutes at 118 C, cool

TABLE 1 Effect of normal human serum on folic acid recovery

Sample no	SFAA before adding folic acid (mμg/ml)	Calculated final SFAA with 100 recovery (mμg ml)	Observed final SFAA (mμg ml	Recovery % of total calculated SFAA
1	0 72	0 81	0 98	121
2	0 80	0 86	0 73	85
3	2 53	1 76	1 83	104
4	2 53	1 50	1 71	114
5	2 48	1 74	1 74	100
6	2 48	1 60	1 66	104

rate normal controls are not found outside the stated normal range during any season or at any age

The patients who had eaten had significantly higher SFAA values than the fasting controls ranging from 5 6 to 12 9 mμg/ml with a mean of 7 9 S D 2 14 and S E 0 54 mμg/ml

Drugs a d radiation

Table III shows that antibiotics in therapeutically effective concentrations inhibit the growth of the test organism

Because of low values often found in leukemia (13 18) the effect of busulfan was studied and because of findings in rheumatoid arthritis that of chloroquine phosphate Neither showed any inhibiting effect (table IV) Sulfaguanidine was also studied but did not seem to inhibit growth Obviously folic acid antagonists inhibit growth completely and in none of some 10 patients given amethopterin either systematically or in tumor perfusion treatment could any growth response at all be found in venous blood

During the period studied the SFAA decreased in 7 of the 9 patients receiving radiotherapy who had more than 2 values and increased in 2 However regression

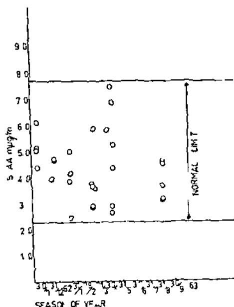


Fig 4 SFAA values from different normal controls during 10 months At no time are values found outside the normal range

coefficients differed from zero in a statistically significant fashion only in 2 of the (decreasing) patients The maximum decrease was 10 3 per cent daily and the maximum increase was 1 5 per cent daily The mean of all values (fig 5) decreased significantly 1 23 per cent per day Whether the decrease is due to radiation or to tumour development is not known

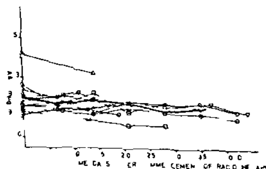


Fig 5 SFAA in 12 tumour patients receiving radiotherapy Heavy line indicates mean of all values.

TABLE III Effect of antibiotics on SFAA assay

Drug tested[1]	Concentration in 4 ml/medium[2]	Growth inhibition[3] (%)
Chlortetracycline	50 μg	100
Tetracycline	50 μg	100
Chloromphenicol	30 μg	100
Streptomycin	50 μg	78 7
Oxitetracycline	50 μg	100
Penicillin	20 I U	100
Methicilline	30 μg	100
Erythromycine	30 μg	100

[1] Paper discs impregnated with the drug (prepared by Central Bacteriological Lab Karolinska hospital Stockholm) added to the medium 2 hours before inoculation

[2] 4 ml of single strength assay medium (21) supplemented with 200 μμg of folic acid was used for the assay

[3] Growth compared with a suitable control to which no antibiotic was added

accessible to lactobacilli It is also possible that other growth inhibitors, or non-specific growth stimulants, are present in the serum Recovery studies, however, did not indicate that serum could inhibit assays, either by binding or otherwise (table I) Nor does the comparison of the L casei growth response in serum and in folic acid solutions indicate the presence of either inhibiting or stimulating factors in the serum, other than folic acid (fig 2)

Normal values

In the fasting controls, the mean SFAA was 4 60 mμg/ml, with a S D of 1 364, a S E of 0 201 and a range of 2 5—7 4 mμg/ml Since the logarithms of the values appeared to be closer to a normal distribution than the values themselves (fig 3), a logarithmic transformation was used in calculating the normal range Thereby, two S D being allowed, the lower limit obtained was 2 4 mμg/ml Of some 50 healthy fasting controls studied since the conclusion of the first series, only one (2 1 mμg/ml) fell outside the established normal range"

No significant change of SFAA with age could be found in healthy adult controls (table II) Nor could any obvious seasonal variations be detected (fig 3) in the present material At any

TABLE IV Effect of chemotherapeutic agents on SFAA assay

Drug	Drug concentration	SFAA without drug (mμg/ml)	SFAA with drug (mμg/ml)	SFAA with drug in % of SFAA without drug
Chloroquine phosphate	3 33 mμg/ml serum	4 74 5 42	4 65 5 61	98 103
Busulfan	4 μg/ml serum	5 70 3 73	7 17 3 36	119 90
Sulfaguanidine	0 6 mg/ml assay medium	[1]0 05	0 05	90

[1] 200 μμg FA added to 4 ml assay medium

Whether, like iron, folic acid is concentrated in the tissues, and thus secondarily low in the serum, or whether there is a true folic acid deficiency in these diseases, remains to be shown There are some indications (18, 19) for the former possibility Findings of low SFAA in states with increased blood cell formation (15, 20) also suggest increased consumption

The promptness with which SFAA reacts to small changes in nutritional status such as those mentioned may make it an important research tool in the *study of the influence on nutrient metabolism in man*

Summary

The serum folic acid activity assay (SFAA) used is described

1 The experimental error is 14 6 %
2 Human serum does not seem to stimulate L casei growth non specifically or to inhibit it
3 Normal fasting human controls had SFAA 2 4—7 4 mμg Previous normal values are reviewed
4 No age dependence of SFAA could be found in non hospitalized normal adult human subjects
5 Food increases SFAA appreciably patients must be fasting for study
6 Most antibiotics inhibit growth but sulfan chloroquine and sulfaguanidine do not
7 During radiotherapy SFAA fell 1 23 per cent per day in patients with malignant tumors

Acknowledgements

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TABLE V Normal SFAA values previously found

Author	State of patient Fasting	drugs	No of subjects	SFAA mμg/ml serum	Whole blood FAA mμg/ml whole blood
Hansen (9)	N i	N i	N i	3 7–9 3	26–118
Herbert (10)	Yes	N i	10	7 5–24	–
Toennies (22)	—	—	100	–	12–150
Grossowicz (8)	—	—	43	–	Average 89
Waters (23)	>2 hrs after food	N i	100	5 9–21	–
Cooper (2)	—	—	100	>6	–
Rachmilewitz (16)		N i	43	3 5–15	47–149

N i = not indicated

Discussion

The present normal values are lower than some reported previously (table V). Herbert (10) had a lower normal limit of 7 5 mμg/ml in fasting patients. Waters et al (24) found a lower limit of 5 9 mμg/ml, but admitted that their patients had eaten 2 hours prior to the assay. Other authors (3,8) had values compatible with those now found, 3 2 and 4 0 mμg/ml, respectively. Although difficult to explain, differences in bioassay normal values between different laboratories are not uncommon.

The present finding of SFAA age-independence is not quite in agreement with values by Hansen et al (9) who found lower whole blood FAA in "a fairly large number" of hospitalized patients between 64 and 87 years old than in normal controls, but does agree with previous findings in cancer patients (18).

Food clearly affects SFAA and only fasting persons should be studied.

The present studies show that all sorts of therapy must be considered when judging SFAA values. Low values found in patients with leukemia and rheumatoid arthritis, however, do not seem to be secondary to drugs.

It is recognized that the nature of SFAA is not yet definitely known. Tentatively it has been identified as a monoglutamate, N-5 methyltetrahydrofolate (11). It is also recognized that bioassays involve appreciable errors. Nevertheless, the reproducibility of the standard curves seems to be sufficiently good, the errors sufficiently small, and serum sufficiently free of non specific inhibitors or stimulants, to allow of the conclusion that the low values found in some disease states, where folic acid deficiency would not be expected, are due to true variations in SFAA rather than to artefacts inherent in the assay.

Reasons for these unexpectedly low values remain to be delineated. In patients with rheumatoid arthritis or malignant tumours an accelerated plasma clearance has been demonstrated (19).

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Incidence of Gastric Cancer in Medically Treated Patients with Gastric Ulcer

By

VAGN RONNOV JESSEN[1] PREBEN AHLGREN and CARL FRISTRUP QVIST

It has been known for several years that if one follows patients diagnosed clinically and radiologically as having gastric ulcer sooner or later one will discover gastric cancer in some of these patients The incidence of gastric cancer varies greatly from study to study, however it averages about 3 % or under in studies involving primarily medically treated patients (4 7, 17 19)

In two recent Danish studies covering large groups of patients treated medically, the incidence of gastric cancer was disclosed to be 4 5 % and 2 8 % These patients were followed for the most part for more than 5 years (1, 13) In three earlier smaller Danish studies the incidence of gastric cancer was from 2 5 % to 5 % (10 11 16) The incidence of cancer reported in studies originating from departments of surgery, especially American is often much higher Ranges from 10 % to 20 % are not unusual (6, 15, 20)

Some of the patients treated surgically for gastric cancer have previously been treated medically for gastric ulcer Koster (12) found this to be true in 95 of 485 patients Stewart (18) concluded, on the basis of the histologic investigation of 1500 stomachs removed for gastric ulcer or cancer that approximately 18 % of the malignant ulcers had originated from simple chronic gastric ulcers It has now been sought to determine the incidence of gastric cancer in patients discharged from medical services with the diagnosis gastric ulcer

Material and methods

The original material for this study comprised all the patients discharged between Jan 1 1947 and Dec 31 1953 from the three departments of internal medicine at the Copenhagen County Hospital Gentofte Denmark with one or more of the following diagnoses ulcus corporis ventriculi ulcus prepyloricum ulcus juxtapyloricum haematemesis or me

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TABLE III Age and sex in 278 cases of gastric ulcer

Age	Males No (%)	Females No (%)	Total M + F
–29	4 (2)	4 (4)	8
30–39	17 (10)	10 (10)	27
40–49	59 (34)	30 (28)	89
50–59	44 (25)	25 (24)	69
60–69	32 (19)	21 (20)	53
70–79	14 (8)	13 (12)	27
80–	3 (2)	2 (2)	5
Total	173	105	278

Ratio Males/females 1 65

TABLE IV Treatment and fate in 278 cases of gastric ulcer

Follow up period (years)	Resected	Not resected		Unknown
		Alive	Dead	
0–1	62		10	
1–3	22		13	
3–5	12		11	
5–	17	112	18	1
Total	113	112	52	1

least a five year follow up in all the cases Patients were traced with the aid of the Danish population registries If an individual had died *during* the follow up period the death certificate and in some cases the cancer registry's file were examined If death had occurred while the patient had been hospitalized the hospital record and if possible the autopsy report were evaluated If death had occurred outside the hospital as much information as possible was obtained from the patient's private physician

Those living at the time of follow up were asked to answer a written series of questions concerning their condition possible hospital admissions and especially the performance of any operations With the use of the hospital records, information was obtained as to the operative findings and the histologic diagnosis Those patients not operated upon were asked to submit to out patient radiologic studies of the stomach a considerable number did respond to this request

Results

It was possible to obtain information on all but one patient or a 99 6 % follow up The results of follow up are given in tables IV to VII 113 patients had been subjected to gastric resection and the length of time between the original diagnosis and operation is given in table IV Eight of the patients were found to have

TABLE I 198 patients excluded from the material, and the reasons for exclusion

Patients with perforated ulcer Perforation in pylorus or with uncertain site	46
Diagnoses ascertained by autopsy only	16
Diagnoses not verified by X ray or operation	6
Healed gastric ulcer or suspicion of gastric ulcer	39
Duodenal ulcer	19
Radiological findings uncertain (pyloric or duodenal ulcer)	38
No ulcer at X ray	33
Original description ulcer — however considered cancer on review No ulcer niche	1
Total	198

TABLE II 278 Patients included in the material

Niches on lesser curvature at least 5 cm from pylorus	221
Prepyloric ulcers (less than 5 cm from pylorus)	30
Niches in other places in the stomach	13
Patients operated for perforated gastric ulcer during the last few weeks	14
Total	278

haena Also included were patients transferred to the medical departments for diet therapy after surgery for perforated gastric ulcer (gastrorraphy)

All of the medical records were thoroughly reviewed by one of the authors (R J) The radiologic studies were reviewed by the other two authors (A and Q) without knowledge of the case histories Only patients with either clear cut surgical or radiologic evidence of gastric ulcer were included There were 476 patients in the original material 198 cases were discarded for reasons given in table I It is of special interest that it was found necessary to exclude a patient obviously originally misdiagnosed as having a gastric ulcer On review of the case there was no question but that the diagnosis was gastric cancer Also excluded were 16 patients who died during the admission In none of these 16 patients had radiologic examination of the stomach been carried out, and in several of them the gastric ulcers disclosed at autopsy were purely incidental findings

Thus, the final case material included 278 patients (table II) The diagnosis was new in 227 patients, whereas in 51 the diagnosis of either gastric or duodenal ulcer had previously been made The diagnosis was made radiologically in 264 cases, and in all of these a distinct niche was demonstrated Ulcers situated 5 or more centimetres from the pylorus were classified as ulcers of the body of the stomach, corpus ulcer, whereas those situated closer to the pylorus were called prepyloric There were 234 patients with ulcers of the body of the stomach, and in 221 of these patients the ulcer was located on the lesser curvature In the remaining 13, the ulcer was situated elsewhere — as a rule on the posterior surface The ulcer was located on the greater curvature in two cases There were 30 patients with prepyloric ulcer Only those cases were included where one could be sure that the ulcer niche was located proximal to the pylorus

Of the 60 patients with perforated gastric ulcer all those were eliminated where the perforation was situated near the pylorus, because it can be difficult to determine the exact site of such perforations Among the 14 patients included in the series only two had prepyloric perforations The others had ulcers of the body of the stomach

The distribution of the patients according to age and sex is given in table III There was a significant preponderance of men, the ratio of men to women being 1 65 The preponderance was not however as great as that of several other recent studies (1 4 13 14) More than half of the patients were between 40 and 60 years of age and approximately 1/8 were under 40 This distribution agrees well with other reports (1 4 13)

Follow up examinations on the 278 patients included in the study were carried out between 1959 and 1961 Thus there was at

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TABLE V Results of follow up examination in 112 living non operated patients

Results of X ray examination	No of cases
Lesser curvature ulcer	10
Prepyloric ulcer	5
Duodenal ulcer	7
Deformed duodenal bulb	5
Sequels from gastric ulcer	9
Normal stomach	30
Total	66
Symptoms in cases not examined by X ray	
No dyspeptic symptoms	36
Mild dyspectic symptoms	10
Total	46

TABLE VI Causes of deaths in 29 cases not autopsied

Diagnoses	No
A No suspicion of gastric cancer	
Pulmonary cancer	2
Mediastinal tumour	1
Malignant neurinoma in nasal cavity	1
Multiple myeloma	1
Suicide	2
Rectal cancer	1
Uraemia (pyelonephritis)	2
Cerebral vascular accident	3
Atherosclerotic heart disease	8
Total	21
B Patients with gastric or hepatic disease	
Gastric cancer	2
Pulmonary tuberculosis haematemesis	1
Debilitas, gastric ulcer	1
Perforated gastric ulcer	1
Hepatic cancer?	1
Chron bronchitis, gastrectasia distended duodenal loop	1
Hepatic coma	1
Total	8

gastric cancer and one lymphosarcoma The most important findings (table VII) will be discussed later Among the remaining 104, no evidence of gastric cancer was found Histologic examination of the resected material was carried out in all but two cases

Gastric resection was not performed in 164 cases Of these, 112 had lived long enough to permit a five year follow up, and in 66 of these cases a radiologic study of the stomach was obtained As is shown in table V, an ulcer niche was found in the stomach in 15 cases and in the duodenum in 7 In no case was there any suspicion of gastric cancer Forty-six of the patients had no wish to undergo X-ray studies, either because they felt well (36 cases) or because they had only minor dyspeptic complaints (10 cases) It may be taken for granted that none of these cases had cancer at the time of the original admission It is likewise unlikely that any of them had gastric cancer at the time of the follow up study, however this cannot, of course be completely ruled out

Fifty-two of the non operated cases were dead at the time of the follow-up investigation The length of time between hospital discharge and death is given in table IV An autopsy was performed in 23 cases, and in none of them was there the slightest evidence of gastric cancer Gastric ulcer was found and verified by histologic examination in 10 cases

In 29 cases no autopsy was performed The causes of death are recorded in table VI A & B There was nothing to suggest gastric cancer in the 21 cases covered in table VI A On the other

hand, the 8 patients listed in table VI B deserve closer consideration Two of the patients had inoperable gastric cancer verified by biopsy their data are listed in table VII (patients nos 2 & 6) The diagnosis of gastric cancer could not be ruled out in three cases even though there was nothing that particularly sug gested it

One was a patient with pulmonary tuber culosis who died 3 years after discovery of gastric ulcer During the last few months of her life she complained of epigastric pains In addition there was coffee grounds vomitus on *2 or 3 occasions No radiologic studies* were carried out during this period There was no palpable abdominal mass Autopsy was not performed

Another was a debilitated patient with a gastric ulcer The patient died at home 13 months after the original radiologic demon stration of a typical gastric ulcer of the lesser *curvature It was not possible to obtain* further information

The third patient was an 82 year old man who died in shock in a surgical ward with the symptoms and signs of a perforated ulcer X ray examination had demonstrated a gas tric ulcer three months previously Permission for autopsy was not given

In three cases it was unlikely that one was dealing with a gastric cancer

One was the patient with questionable hepatic cancer Two and one half years be fore death a *lesser curvature ulcer was de monstrated Two later radiologic studies* — one just half a year before death — revealed a completely normal stomach The liver was noted to be large and irregular throughout *the patient's entire course*

Another with gastric atony *and a dilated* duodenal loop died after a 20-month follow up In the course of that period three sepa rate radiologic studies all showed exactly the same features ulcer of the lesser curvature gastric atony and dilated duodenal loop She died one month after the last X ray study of advanced chronic *bronchitis*

The third patient age 70, died three years after radiologic demonstration of an ulcer of the lesser curvature A serious attack of in fectious hepatitis at age 65 developed into *cirrhosis The last pictures* of the stomach were taken 20 months before he died in hepatic coma and 15 months after the diag nosis gastric ulcer had been made At the time of the last study the ulcer crater was much smaller than on the first pictures and there was nothing to suggest cancer

As is obvious from the above, a malig nancy of the stomach was found in 11 cases The diagnosis was verified by gas tric resection in 9 and by biopsy in 2 *Age, sex, duration of symptoms the most* important objective findings and the treatment are listed in table VII One patient had a lymphosarcoma (no 7) *the other 10* had cancer The diagnosis of ulcer was new in patients no 1 to 10 while no 11 had been known to have an ulcer a year before she was included in the study

As is shown by table VII, malignancy was verified within the course of a year in 10 patients In 6 cases (nos 1 3 4, 5 8 9) advanced carcinomatous infiltra tion was found In 4 of these cases a chronic peptic ulcer was also found In 2 cases (nos 2 & 6) the diagnosis was made by examination of biopsy material from metastases and the stomach itself was not examined In one case *(no 10)* histologic examination revealed a chron ic peptic ulcer with local carcinoma tous changes of the rim Lymphosarcoma was found at the base of a chronic ulcer and in the subserosal tissue of one patient (no 7) Patient no 11 was followed for 5 1 2 years with *no radiologic evidence*

TABLE VII 11 cases of gastric tumours The most important signs and symptoms

Case no	Sex/Age		Duration of previous symptoms	Free gastric acid	Hb (%)	ESR (mm/h)
1	♀	38	4 years	+	95	10
2	♂	64	3 years	+	88	4
3	♀	68	2 years	+	94	30–11
4	♂	58	3 months	+	93	5
5	♀	56	1 month	+	90	45
6	♂	38	1 year	?	105	16
[1]7	♂	52	1 year	+	90	?
8	♂	41	1 year	?	70	60
9	♂	58	6 months	+	96	20
10	♂	44	4 years	+	65	7
11	♀	49	7 years	+	81	16

[1] In this case there was no cancer The patient had lymphosarcoma of the stomach

of an ulcer A year later, however, there was a recurrence of ulcer symptoms, and while X-ray studies were interpreted as showing a simple ulcer, a prepyloric cancer was found at operation

A review of the clinical symptoms and the laboratory findings revealed nothing that could clearly separate the cancer patients from the others Their ages varied from 38 to 68 with an average of 51 The average for the study was almost 54 years Duration of the dyspeptic symptoms also failed to provide any sure guide, since in only three cases had the symptoms been present less than a year Among the others, symptoms had been present 1 to 7 years Gastric juice was examined in 9 cases, and free acid was found in all Haemoglobin values and sedimentation rates (Westergren) were likewise of little diagnostic value In only 2 cases was the percentage haemoglobin less than 80, and in 8 of the 10, the ESR was 20 mm/hour or less

Examination of the faeces for blood (benzidine test) gave the sharpest difference between cancer patients and the others (results are not given in table VII) There was melaena or occult bleeding during the entire hospital stay of 39 patients and of these 5 (13 %) (patient nos 1, 2, 3, 5, 6) had cancer There was a series of at least 3 negative benzidine tests in 221 cases Six of these (2 7 %) (nos 4, 7, 8, 9, 10, 11) were later shown to have gastric malignancy — in 3 cases within a month

Discussion

Among 278 patients with gastric ulcer followed between 5 and 12 years, 10 definite cases of gastric cancer and one of gastric lymphosarcoma were found In 3

X ray	Tumour suspicion	Duration from admission to final diagnosis	Treatment
Ulcer on lesser curv	+	9 months	Partial gastrectomy
3 ulcers on lesser curv	+	3 months	Biopsy only
Ulcer on lesser and greater curv	+	4 weeks	Total gastrectomy
Ulcer on lesser curv	+	3 weeks	Partial gastrectomy
Ulcer on lesser curv	+	3 weeks	Partial gastrectomy
Ulcer on lesser curv	+	3 weeks	Explorative lapatomi Biopsy
—	+ (gastrorraphia)	7 months	Partial gastrectomy
Ulcer on lesser curv	—	1 month	Partial *gastrectomy*
Ulcer on lesser curv	—	1 year	Partial gastrectomy
Ulcer on lesser curv	—	1 month	Partial gastrectomy
Ulcer on lesser curv	—	$6^1/_2$ years}	Partial gastrectomy

additional cases the possibility of gastric cancer could not be ruled out, even though there was nothing that strongly suggested it Thus in this series there was a cancer incidence of 3 6 % or — if one wishes to include the uncertain cases — of 4 7 %

Without doubt there were more cases of gastric cancer in this series than one would *expect* to *find in* a comparable group followed for the same period of *time This summarising statement* obviously does not allow one to draw any conclusions as to the possible relation ship between benign and malignant gastric ulcer

There are two ways to proceed if one wishes to determine whether ulcer predisposes to cancer One method — *trying to* determine from pathologic material whether a cancer developed from a pre-existing ulcer — is probably of questionable value It will always be difficult to decide which *one of the* lesions was *primary* or whether one is dealing with two independent diseases Moreover, the findings will always be rather dependent on the time at which the stomach is examined The other method the surer one demands a long period of observation and an energetical ly conducted investigation One must exclude all those patients dying or being operated upon within that period of time in which — *if a cancer is found — one can be reasonably* sure that it was present at the time the ulcer diagnosis was made In addition one must also exclude those cases where the diagnosis of cancer was made in any other way during the same period of time If one finds a signifi cant increase in the incidence of cancer in a series of patients collected using the above *criteria then it should* be permissible to con clude that gastric ulcer predisposes to gastric cancer On the other *hand* one must be aware of the fact that if one does not find an increase in the incidence of cancer *in such a group then one may only conclude that* gastric ulcer does not predispose to gastric can cer provided that one can be sure that the excluded cases of cancer did not develop during the follow up period

TABLE VII 11 cases of gastric tumours The most important signs and symptoms

Case no	Sex/Age		Duration of previous symptoms	Free gastric acid	Hb (%)	ESR (mm/h)
1	♀	38	4 years	+	95	10
2	♂	64	3 years	+	88	4
3	♀	68	2 years	+	94	10–11
4	♂	58	3 months	+	93	5
5	♀	56	1 month	+	90	45
6	♂	38	1 year	?	105	16
7[1]	♂	52	1 year	+	90	?
8	♂	41	1 year	?	70	60
9	♂	58	6 months	+	96	20
10	♂	14	4 years	+	65	7
11	♀	49	7 years	+	81	16

[1] In this case there was no cancer The patient had lymphosarcoma of the stomach

of an ulcer A year later, however, there was a recurrence of ulcer symptoms, and while X-ray studies were interpreted as showing a simple ulcer, a prepyloric cancer was found at operation

A review of the clinical symptoms and the laboratory findings revealed nothing that could clearly separate the cancer patients from the others Their ages varied from 38 to 68 with an average of 51 The average for the study was almost 54 years Duration of the dyspeptic symptoms also failed to provide any sure guide, since in only three cases had the symptoms been present less than a year Among the others, symptoms had been present 1 to 7 years Gastric juice was examined in 9 cases, and free acid was found in all Haemoglobin values and sedimentation rates (Westergren) were likewise of little diagnostic value In only 2 cases was the percentage haemoglobin less than 80, and in 8 of the 10 the ESR was 20 mm/hour or less

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Discussion

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ulcer patients and in these the cancer diagnosis was *proved* within the first few months No new case of malignancy was found among the remaining patients during the *next three* years On the other hand Cain et al 's series (5) from the Mayo Clinic gives an entirely different picture *Among* 414 ca es of primarily medically treated gastric ulcer, there were 43 cases of gastric cancer The diagnosis was proved *after more than one* year in 24 cases and after more than 3 years in 17 cases The frequency of mistaken diagnosis was thus 19/414 or 4 6 % This is not essentially different from that reported in the three Copenhagen studies However there was a considerable incidence of cancer in the later course of these patients — much greater than in the Danish studies The reason for this is not clear

The important question is of course, how many of these medically treated ulcer patients actually have cancer at the time they are first admitted for treatment and how many of these cancer patients are inadequately treated

The attitude toward surgical treatment was rather conservative in the three Copenhagen series in that only about 2 5 ths of the cases were referred for gastric resection during the follow up period Thirty cases of gastric cancer were found among the 813 patients Among these the diagnosis was made in 24 within the first year of follow up Seventeen of the 24 were *transferred* directly to a surgical ward with the thought of gastric resection In all 314 patients were sooner or later subjected to gastric resection If one had adhered to *the principle that because of the risk of* cancer all cases of gastric ulcer should be operated on, one would have found among the remaining 500 patients 7 cases of cancer and perhaps prevented another six — a total of 13 cases or 2 6 % Such a rigid attribute cannot be defended, because the mortality for gastric resection is still around 2 % (8) and the *morbidity in many* cases is not insignificant, and finally because one cannot hope to cure more than 50 % of the operable ulcer like gastric cancers (2)

The surgeons maintain that the mortality with segmental resection is less than 2 % (9) and with local excision of a gastric ulcer less than 1 % (3) It should however be borne in mind that especially the later method has a questionable effect in preventing cancer and that these methods can be used only in hospitals possessing a specially trained pathologist who easily masters the frozen section technique

It follows from the foregoing that the results of conservative therapy as practised in these three groups has not been completely unsatisfactory however there still exists room for improvement

By reviewing the radiologic studies we have tried to separate the patients into two groups Group I contained those patients where neither X ray nor gastrorraphy for perforated ulcer suggested gastric cancer It was possible to obtain information about 255 of the 256 patients in this group Three were shown to have gastric cancer at operation Two were transferred to a surgical ward after a period of observation (table VII nos 8 & 10) because of the *severity of their* symptoms Wide spread cancer was found at operation a year later in a third

It is our opinion that one year is an adequate period of time As mentioned above, in 9 out of 10 of our cases the diagnosis of gastric cancer was made within a year, and in 7 cases it was actually made in less than 3 months Undoubtedly these 7 already had cancer at the time the ulcer diagnosis was made This probably applies also to the last 2 since they had wide-spread cancers at operation performed 9 months and one year later As shown in table IV, gastric resection was undertaken in 62 of our cases within the course of the first year Ten of the non resected cases died within the same period, and it was in 2 of these that a cancer diagnosis was established by biopsy The series was in this way limited to 206 cases Fifty one were later subjected to gastric resection, and one case of gastric cancer was found 6 1/2 years after the original diagnosis Among the non operated cases 112 allowed a 5 to 12-year follow up Not a single case of even suspected cancer was found Finally, there were the 42 deaths No cancer was found, but in two cases this diagnosis could not be completely ruled out Thus, among these 206 patients only one proven case of gastric cancer developed during the follow up period

If Lyngborg and Nielsen s material (13) is treated in the same way one finds that in 4 of the 7 cases cancer had undoubtedly been present from the beginning of the period of follow up This means that among 156 non resected cases cancer developed in 3 after 2, 3 and 5 years in the course of the 5 to 15 year follow up The resected cases are not considered, because no information is given as to when surgery was performed It is mentioned, however, that cancer was not found in any of these cases

The third series from Greater Copenhagen comprised 287 patients (1) Ninety five cases were transferred to a surgical ward soon after admission and there underwent gastric resection, 10 cases of gastric cancer were found 183 of the remaining 192 patients were followed from 0 to more than 10 years In two-thirds of the cases the follow up was for more than 5 years Twenty three of these patients later underwent gastric resection, however, they will be excluded from consideration, because the date of the surgery is not known None of them had cancer Among the remaining 160 patients, cancer was found in three In one of these, the diagnosis was made after only one year from the date of the original diagnosis, so that it is probable that the patient already had cancer at the time he was included in the series Only in the 2 cases where cancer was found after a course of 2 and 6 years, respectively, is it justified to conclude that cancer developed during the follow up period

It is obvious from the above that not quite the same criteria were used in selecting the groups of medically treated ulcer patients in these three almost simultaneous Danish studies It is, however, also obvious from the above that in none of the series was clear evidence presented that indicated that gastric ulcer predisposes to gastric cancer In the three specially limited studies comprising approximately 500 patients, mostly followed for more than 5 years 6 cases of cancer are presented or a little over 1 % It is questionable whether this is a greater incidence than one would find in a group of normal persons of the same age and sex

There are only a few of the non-Scandinavian studies that provide enough information to answer the same question Doll et al s work (7) agrees with the above in that these authors found a cancer incidence of 5 among 285

viously undergone medical ulcer therapy (12) That statement hardly seems to hold for the medical materials just mentioned *unless we take* into account the patients transferred to a surgical ward for operation after a period of observation and dietary treatment in a medical ward

The incidence of cancer in these medical series, *i e 3 to 4 %, is* decidedly lower than that usually reported from surgical departments The reason is, undoubtedly, that both cover selected materials Patients with severe symptoms will often be admitted directly to a surgical ward In other cases, radiologic studies of the stomach will be done on an out patient basis before the admission (in the present series this was true in about 20 % of cases) and if the studies show pyloric stenosis or if cancer is suspected the patient will be admitted directly to a surgical service Finally, the fact must be remembered that some of the medical patients are transferred to a surgical service on suspicion of cancer Marshall and Welch's work from the Lahey Clinic (14) illustrates this Among 131 operated ulcer patients the incidence of cancer was 19 8 % However these cancer patients comprised only 3 3 % of the total number of patients treated for gastric ulcer during the same period of time at that hospital The length of the follow up period is not mentioned Smith et al s work (17) also shed light on the same problem Eighty-one of 578 primarily surgically treated patients had gastric cancer while only 7 of 422 primarily medically treated patients were found to have gastric cancer Some of the patients however were followed for only two months

It is, therefore, understandable that, for the surgeon, the risk of cancer is probably the most important consideration in the treatment of gastric ulcer It also explains why many surgeons on their experience advise surgery as the treatment of choice

It should, however, be just as obvious that the problem is of much less importance for the internist The majority of patients with cancer will be operated primarily if the practice is to operate on those patients where the radiologic evidence *suggests cancer and/or where the* symptoms themselves indicate surgery The incidence of cancer among the non operated patients will, as a rule, be low, and therefore, one cannot be justified in recommending routine surgery for this group We do not wish to indicate that results are satisfactory however as far as the internist is concerned, they can be improved with the use of better diagnostic procedures, especially repeated radiologic studies performed by a competent radiologist cytologic examination of gastric juice, the augmented histamine test gastroscopy and long series of benzidine tests

Finally one should not overlook the fact that if all medical patients with gastric ulcer were routinely *transferred* to a surgical service then the basis for active surgical therapy would be affected to some degree since in this way the incidence of cancer in surgical series would be reduced

Conclusion and summary

A series of 278 patients treated for gastric ulcer under medical auspices at the

patient (no 9) One may, therefore, conclude that the original ulcer diagnosis was erroneous in 3 cases or 1 2 % Of the 3 questionable cases that died 3, 13 and 36 months after diagnosis, the first 2 may have been erroneously diagnosed When these 2 cases are added to the previous 3, the percent of possibly erroneously diagnosed cases comes to 2 % The patient operated upon for gastric cancer 6 1/2 years after the ulcer diagnosis was made is also in group 1, but cannot of course, be considered a case of mistaken diagnosis

Group 2 comprises the 22 patients in which radiologic studies (21 cases) or surgical findings (1 case) suggested gastric cancer Six had cancer and one had lymphosarcoma The diagnosis was made at operation in 5 cases biopsy from metastasis in 2 (table VII nos 2 & 6), gastric resection in the other 3 (nos 3, 4, 5) One patient (no 1) was first operated on about 9 months after the first studies because of uncharacteristic radiologic findings, however, on review of the films, one was almost sure that the changes were caused by cancer The operative findings *in patient no 7 were quite suggestive of* malignancy, but biopsy from the edge of the perforation showed only chronic peptic ulceration It was only later that the diagnosis lymphosarcoma was made None of the remaining 15 had gastric cancer Six underwent gastric resection, 3 died of intercurrent disease and were autopsied The other 6 did not undergo surgery, but repeated radiologic studies in 4 of them failed to suggest cancer The other 2 of the 6 felt well, and therefore refused to submit to radiologic studies Thus in this group, there was an incidence of malignancy of 32 % and of cancer of 27 %

If all of the suspected cases had been submitted to surgery and if the indications for surgery had not otherwise been changed, one would have found 8 of the 9 cases of cancer that had been there from the beginning Thus, there would have been only one case of untreated cancer among this series of 278 cases of gastric ulcer

Doll et al 's series (7), which was about the same size as the present one, was classified in a similar way There was, however, a difference in the composition of the two groups in that Doll et al included only patients with newly diagnosed gastric ulcer The series comprised 285 cases followed for 3 years Among the 266 patients in whom initially no suspicion of gastric cancer, one patient died of that disease in the course of a few months None of the others presented any sign of gastric malignancy during the period of follow up, however, 10 cases were lost track of Cancer was suspected in 19 and confirmed in 4 In 3 of them this happened within the first two months *and in the fourth after 10 months* The incidence of cancer was 0 4 % in the first group and 21 % in the second The authors concluded that in the majority of cases benign gastric ulcer can be differentiated from gastric cancer with sufficient accuracy, and that the risk of diagnostic error is not great enough to justify surgical treatment of gastric ulcer as a routine procedure

As has already been mentioned, some surgeons maintain that a considerable number of the patients who come to operation for gastric cancer have pre

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A series of 278 patients treated for gastric ulcer under medical auspices at the

Copenhagen County Hospital in Gentofte is presented. The diagnosis in 264 cases was made radiologically by the demonstration of a niche in the gastric wall, and in 14 cases at operation for the suture of a perforated gastric ulcer.

Men predominated, the ratio of men to women being 1.65. In agreement with similar studies, we observed that gastric ulcer was a disease of the middle aged and the elderly, 7/8 of the patients were over 40 years of age.

Follow-up studies were made after a period of 5 to 12 years, and it was possible to locate 99.6 % of the cases. Ten cases of gastric cancer and one case of lymphosarcoma were disclosed. Only in one case it was quite certain that cancer had developed during the follow up period. In the other 9 patients, cancer had presumably been present from the beginning of the study. The diagnosis of cancer was made within 3 months in 7 patients and within a year in the other 2.

There were no distinguishing characteristics that clearly separated the cancer patients from the others. There was no difference in age, duration of symptoms, haemoglobin concentration or sedimentation rate. Gastric juice was examined in 9 of the cancer cases, and free acid was found in all 9. Daily examination of the faeces using the benzidine test gave a consistently or intermittently positive reaction in 5.

The results from this study are compared with those from two contemporary Copenhagen studies comprising about the same number of patients followed for about as long. The incidence of gastric cancer was from 3 to 4 %, an incidence undoubtedly higher than one would expect to find in a comparable group of normals. *Those patients in whom the final diagnosis was made at operation within the first year and those patients who died within the first year were not included in the study.* In this way it was possible to separate those groups of patients where one—if cancer were found—could be reasonably sure that cancer had been present from the beginning of the *follow-up period.*

Our series was limited in this way to 206 patients, and one case of cancer was found. If all three series were considered in this way, one finds 6 cases of cancer among approximately 500 patients. This modest cancer incidence cannot be taken as evidence that gastric ulcer truly predisposes to gastric cancer.

Thirty cases of gastric cancer were discovered during follow up in the 813 patients in the three studies. Twenty four patients are considered to have had gastric cancer from the start and 17 were transferred directly to surgery. 314 of the 813 patients were sooner or later subjected to gastric resection. If the remaining 500 patients had been operated on, 7 additional cases of cancer would have been detected and maybe one would have been able to prevent another 6. Since there still is a certain operative risk, and since there is a not insignificant post-operative morbidity and since one can only hope to cure about 50 % of the operable malignant ulcers, we concluded that such a rigorous attitude can hardly be justified with regard to gastric ulcer patients admitted to a medical service. Local excision of gastric ulcers recommended by many surgeons, is possible only in hospitals where reliable facilities

and personnel are available for frozen section diagnosis

Doll et al s work and the present study both suggest that if one subjects to surgery all the medical patients who are under suspicion for cancer, after a thorough X ray examination by a competent radiologist and likewise all these patients in whom the very severity of the symptoms indicates surgery then almost all the patients with gastric cancer will primarily be operated on

The frequency of cancer is lower in medical series than in surgical because a certain selection takes place before admission This explains why many surgeons on principle operate on all cases of gastric ulcer while internists often favour a more conservative approach

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From Medical Department P (Head J Hess Thaysen, M D) Rigshospitalet Copenhagen Denmark

Intra-vitam Diagnosis of Oxalosis

By

Jørgen Lindholm

Oxalosis is a rare disorder The first definite case was described less than fifteen years ago and since then only 33 cases have been reported in the literature Most of these were diagnosed at autopsy

The purpose of this paper is to present a typical case and to report the means by which the diagnosis was established in vivo

Case report

The patient was a 19 year old male born 1945 admitted to the Medical Department P University Clinic of Copenhagen in July 1964 on account of severe uremia

The family history revealed that the maternal grandfather — who was still alive — and the maternal greatgrandfather both had suffered from renal calculi

The patient had been well until the age of 3 years apart from a herniotomy when he was 18 months old

When he was 5 the first renal colic occurred and was in the ensuing years followed by numerous other attacks Thus at the age of 9 years 14 stones were discharged in the course of 6 months

In 1955 at the age of 10 he was admitted for the first time to hospital A urinary infection was discovered and X ray examination revealed a stone in the parenchyma of the right kidney

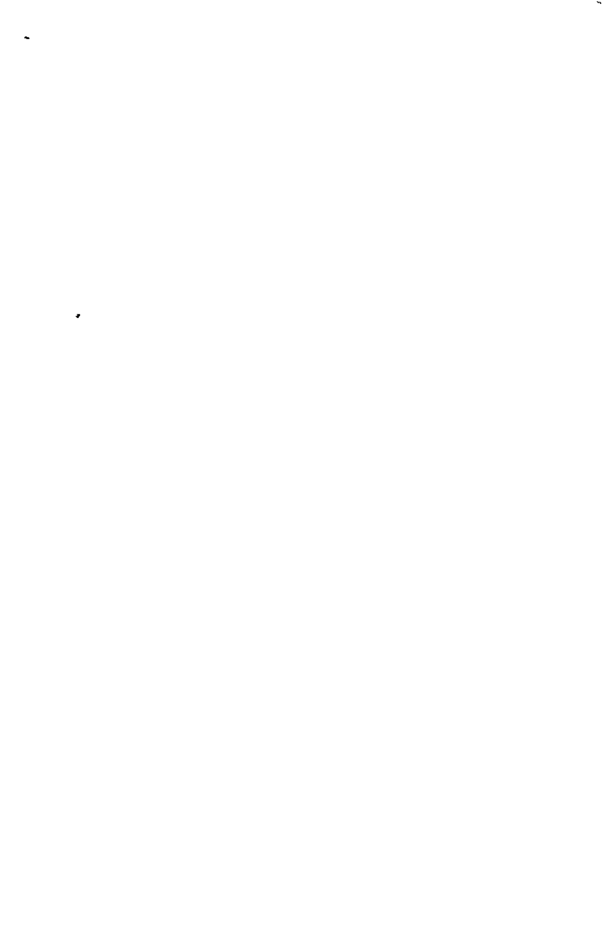
During the following years more calculi were passed, and in 1957 he was readmitted for surgical removal of a stone in the urethra X ray examination now showed a big calculus in the right kidney

After more attacks of renal colics and two episodes with pyelitis the patient again entered hospital in 1959 because of a new stone in the urethra This stone was removed and chemical analysis showed it to consist of 95 % calcium oxalate and 5 % calcium phosphate Serum calcium and serum phosphate were 10 6 mg and 5 6 mg per 100 ml respectively X ray examination of the bones was normal Intravenous pyelography showed several stones on both sides delayed excretion from the right kidney and no excretion at all from the left kidney

For years thereafter there were no symptoms from the urinary tract and the patient was able to manage his job as a seaman

In the summer of 1964 slight edema developed together with malaise and headache but there were no urological complaints He was admitted to hospital for the third time Proteinuria together with a grave anemia and azotemia were discovered The hemoglobin was 45 % and serum creatinine 35 mg/100 ml Because of a rapidly increasing serum potassium level the patient was referred to

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In 1955 at the age of 10 he was admitted for the first time to hospital A urinary infection was discovered and X ray examination revealed a stone in the parenchyma of the right kidney

During the following years more calculi were passed, and in 1957 he was readmitted for surgical removal of a stone in the urethra X ray examination now showed a big calculus in the right kidney

After more attacks of renal colics and two episodes with pyelitis the patient again entered hospital in 1959 because of a new stone in the urethra This stone was removed and chemical analysis showed it to consist of 95 % calcium oxalate and 5 % calcium phosphate Serum calcium and serum phosphate were 10 6 mg and 5 6 mg per 100 ml respectively X ray examination of the bones was normal Intravenous pyelography showed several stones on both sides delayed excretion from the right kidney and no excretion at all from the left kidney

For years thereafter there were no symptoms from the urinary tract and the patient was able to manage his job as a seaman

In the summer of 1964 slight edema developed together with malaise and headache but there were no urological complaints He was admitted to hospital for the third time Proteinuria together with a grave anemia and azotemia were discovered The hemoglobin was 45 % and serum creatinine 35 mg/100 ml Because of a rapidly increasing serum potassium level the patient was referred to

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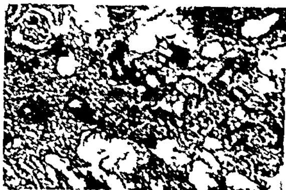


Fig. 1. Microphotography of kidney biopsy under semi-polarized light. Deposits of typical oxalate crystals in tubules together with interstitial fibrosis and marked cellular infiltration.

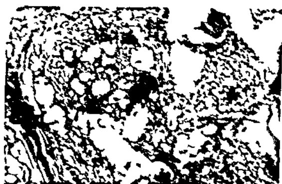


Fig. 2. Microphotography of biopsy from iliac crest under semipolarized light. No foreign body reaction to the oxalate crystals.

the Dialysis Center at Medical Department P, Rigshospitalet, for treatment and further study.

On admittance the patient was in rather poor condition. Besides the anemia and uremia there were grave electrocardiographic changes because of the high serum potassium. B.P. was 145/80. Urinalysis showed proteinuria, hematuria and pyuria. The diuresis was about 150 ml/24 h and creatinine clearance 0.2 ml/min. Serum calcium was low and serum phosphate high — undoubtedly because of the uremia. There was no biochemical evidence of liver disorder. Ophthalmological examination was uninformative.

The patient was hemodialysed three times while further investigations were carried out. These included a plain X-ray examination of the kidneys and retrograde pyelography on both sides. Many calcifications were found in the parenchyma of both kidneys, but none in the pelves or ureters. A renal angiography revealed multiple renal arteries on the left side but normal conditions on the right.

On account of the history of the disease oxalosis was suspected. Needle biopsies were taken from the kidney and the liver and a surgical biopsy was performed from the iliac crest.

The liver showed no changes, whereas the kidney presented heavy pyelonephritic lesions together with an abundance of refractile crystals in the lumina of the tubules throughout the renal parenchyma (fig. 1). They were visible with Nicol prisms, but difficult to recognize with ordinary microscopy. These crystals were morphologically identical with oxalate crystals and X-ray diffraction confirmed that they consisted of calcium oxalate monohydrate. The biopsy from the iliac crest showed entirely the same picture with heavy deposits of oxalate scattered throughout the spongious and medullary elements (fig. 2).

A determination of urinary oxalate showed that the patient excreted about 1 mg oxalate/24 h, but it is to be noted that the diuresis was then 60 ml/24 h and creatinine clearance less than 0.1 ml/min. In comparison with two control patients the spinal fluid revealed a much higher concentration of oxalate by semi-quantitative determination.

On the basis of the above-mentioned findings a diagnosis of oxalosis was made. Since renal functional impairment was severe and irreparable, it was decided to give up further dialytic treatment and death ensued due to progressive uremia.

At *autopsy* two somewhat contracted and pale kidneys were found. The right kidney measured 9 × 5 × 2 1/2 cm and weighed 75 g, the left kidney 11 × 5 1/2 × 3 cm and weighed 110 g. The capsules stripped easily and a gritty sensation was observed when the kidneys were cut. In the parenchyma of the left kidney two rather big and in both kidneys several smaller calculi were found. There was double anlage of the right renal

TABLE 1 Sex distribution and age at onset of symptoms in oxalosis

Age (years)	0–10		11–20		21–30		31–40		41–50		51–60		?		Total	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
No	15	5	2	4	4	0	1	1	0	0	1	0	1	0	24	10
Total	20		6		4		2		0		1		1		34	

pelvis but the rest of the urinary tract was normal and without calculi

There was a moderate degree of left ventricular hypertrophy of the heart but the autopsy otherwise revealed nothing of particular *inErest*

Microscopic examination of the kidneys and bone from the spine showed a picture identical with that of the biopsy specimens and the same crystals were found in the myocardium the walls of hepatic vessels the thymus skeletal muscles and the adipose tissue of the neck None were found in the spleen lymphatic nodules endocrine organs or the central nervous system

Discussion

Oxalosis and primary hyperoxaluria are both disorders of metabolism and both characterized by excessive urinary excretion of oxalate and a strong tendency to formation of urinary calculi Calcium oxalate is deposited in the renal parenchyma and in oxalosis even in other organs Secondary pyelonephritis is the rule and death eventually ensues in uremia

Today oxalosis and primary hyperoxaluria are by most authors considered to be fundamentally identical disorders although no definite proof of this hypothesis has been presented

These diseases are rare Thus it has been possible to collate only 33 reports of oxalosis from the literature (for references see 5), and the total number of cases of both disorders hardly exceeds one hundred

It appears from table 1 that 24 of 34 patients with oxalosis are male and 10 female In 26 cases the first symptoms appeared before the age of 20 i e in childhood or early adulthood The course from onset to death is most variable 4 weeks being the shortest and 22 years the longest It is interesting that several patients have had periods with copious discharge of calculi followed by long intervals with no urological symptoms Further it is to be noted that 4 cases of the 34 had had no urinary stones 3 of these were children and in all 4 the course was very rapid with death following soon after onset of symptoms

Most of the symptoms are due to the urinary stones — renal colics hematuria secondary infection with attacks of pyelitis and development of hydronephrosis or pyonephrosis Nephrocalcinosis visible on X ray is a common feature

Short stature arthritis and cardiac arrhythmias have been reported due to deposits of crystals in the bones joints and myocardium

The pathological changes always include deposits of oxalate in the renal parenchyma — especially in the lumina of the tubules but sometimes also in the

Fig 3 Pathways in oxalate metabolism

glomeruli or interstitially Most other organs may be involved, the myocardium and the bones are the most frequent sites of extrarenal deposits, but also the testes, thymus and thyroid gland are often involved

The crystals are round or angular refractile and easily seen in polarized light They are not stained by hematoxylin eosin, several other methods of staining have been proposed, but they are most readily identified by means of X-ray diffraction

There is remarkably little foreign body reaction to these crystals in the involved organs, but pyelonephritic changes in the kidneys are very common

Biochemically hyperoxaluria is the most important symptom Normally 10—40 mg of oxalate are excreted in the urine per 24 hours but in these patients this value may rise to 400—500 mg Terminally the renal function may be so compromised that normal or even subnormal values may be found If the output of oxalate is compared with creatinine excretion it may still be found that there is a relative hyperoxaluria Also the concentration of oxalate in the spinal fluid seems to be elevated

The pathogenesis has been studied to some extent in recent years

The metabolism of oxalate is shown in fig 3 Glycine and ascorbic acid are both precursors of oxalate It has not been established whether ascorbic acid is metabolized to oxalate directly or via glyoxylate, nor is it known to what extent the metabolism of hydroxyproline plays a role Glycine can be converted reversibly by a transamination and irreversibly by oxidative desamination to glyoxylate, which further can be metabolized to formate + CO , glycollate and oxalate Evidence has accumulated that the disorder is due to a block in the reaction glyoxylate → glycine (4, 5)

The etiology is unknown, but an inborn error of metabolism is suspected Why some cases do not present themselves until relatively late in life is not clear, but the same phenomenon can be observed in other congenital metabolic disorders, e g porphyria

Concurrent oxalosis in two or more sibs has been reported (1, 3 6) At present however, no definite conclusions can be drawn regarding the mode of inheritance due to the scanty information in the literature

Treatment has so far been unsuccessful Since oxalate is only found in in ordinary mixed diet in a small quantity and since less than 5 % of ingested oxalate is absorbed (2) it is obvious that restriction of dietary oxalate would be of no therapeutic significance Reduction of dietary glycine or trapping of glycine with benzoate has been tried in vain (2) Since pyridoxine presumably is a necessary co factor for the conversion of glyoxylate to glycine, treatment with pyridoxine has been attempted but without effect (4)

Summary

A typical case of oxalosis is presented The patient was a 19 year old male, who had been passing urinary calculi in heavy amounts from the age of 5 until 4 years prior to his death in severe uremia at the age of 19 The uremic stage was short and not followed by hypertension Oxalosis was diagnosed prior to death through demonstration of oxalate crystals in biopsies from the kidney and bone, although no hyper oxaluri could be detected — undoubtedly because of the severe renal insufficiency at the time of study The oxalate concentration of the spinal fluid seemed to be elevated At autopsy crystalline deposits were furthermore found in the heart thymus, hepatic vessels, adipose tissue and skeletal muscle X ray diffraction showed that the crystals consisted of calcium oxalate monohydrate

A brief survey of oxalosis is given together with a short discussion of the underlying biochemical disorder and the various attempts at treatment

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From the First Medical Department, Sahlgrenska sjukhuset, and the Medical Department of Molndal, University of Goteborg Goteborg, Sweden

Long-term Prognosis in Essential Hypercholesterolemia

The Effect of Strict Diet

By

B Hood, H Sanne G Orndahl, Marianne Ahlstrom and G Welin

To our knowledge there is a paucity of reports on the long term fate of patients with essential hypercholesterolemia and hyperlipemia who have been thoroughly investigated from a clinical standpoint There is also little information on the long term effect of attempts over a period of years to lower the blood lipid levels with dietary and therapeutic or combined regimens

In their follow up study of Kornerup s (7) material of essential hypercholesterolemia with observation periods from 11 to 13 years Piper and Orild (12) found that 34 per cent of cases had died in the intervening period those dying of coronary occlusion doing so at an aver age age of 47 2 years The average age of the survivors in 1954 was 48 5 years

Nelson (11) found no new cardiovascular accidents in his material of 33 patients on a high protein low fat low cholesterol diet With one exception every patient had such accidents prior to the initiation of therapy Observation times were only 6—14 months No control material was presented Lyon (8) reported a 5 year study of 217 survivors after myocardial infarction 143 of these followed a low fat, low cholesterol diet. The group on diet showed a significant decrease in the ultracentrifugally deter mined β lipoprotein levels or more precisely the atherogenic index according to the Gofman group There were decisively lower rates of recurrence of myocardial infarction and death in this diet group as compared with the non diet group

A study conducted with great persist ence and determination is the one reported by Morrison (10) who in 1946 started to treat a series of 50 cases of myocardial infarction with a low fat, low-cholesterol diet and compared this series with 50 alternate cases who remained on an ordinary diet He reported his results after 3 8 and 12 years, respectively After 12 years, 19 lived in the diet group none in the con

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trol group His diet was a low-calorie one and there was a definite decrease in weight in the treated subjects

Koranyi (6) in a 3 year study of myocardial survivors (and a small number of patients with peripheral vascular disease) found a 8 6 per cent mortality in the low fat group as compared with 19 7 per cent in his control group

Our local interest in this field started with Welin's first studies in the First Medical Department, Sahlgrenska sjukhuset, Goteborg 1946—1947, published 1948 and 1949 (13, 14) However, the studies did not gain real momentum until facilities for lipid measurements on a large scale were made available in 1955 The medical department of Molndal joined in these studies in the latter part of 1954

Clinical material

The clinical material presented in this report includes all cases admitted to these two departments between 1947 and the end of 1958 where at least two cholesterol determinations showed levels above 300 mg % A small number (22) of patients with definite tendinous xanthomatosis, who happened at the initial examination not to fulfil this criterion have also been included Cases of nephrosis, hypothyroidism and manifest diabetes were excluded Since many hyperlipemics in particular occasionally have slight glucosuria, cases with up to 20 of urinary output of glucose showing no signs of ketosis and having no active antidiabetic treatment have been included — as have those who merely demonstrated an abnormal glucose tolerance test

Since the policy of following up the immediate aftercourse in myocardial infarction with repeated lipid measurements was only used to a limited extent during these years, a very great number of patients with lipid elevation must have gone undetected It is our experience, also, that a good number of cases, although showing a series of high blood lipid levels, have near been recorded as essential hypercholesterolemia or essential hyperlipemia and have thus not been located Thus, our material is limited to 458 patients admitted up to the end of 1958 and where we have observation times between 5 and 17 years Of these 124 presented xanthomata It should be observed that owing to some limitations of laboratory resources symptoms from the brain or the lower extremities during the early years did not prompt investigation of cholesterol except in the very young age groups

The asymptomatic group (96 subjects) included subjects where the cholesterol measurement was prompted by the findings of hypercholesterolemia in parents or siblings with/or without vascular symptoms A minor number were those where xanthomata and/or xanthelasmata were found on physical examination It seems therefore conceivable, that if non lipid pathogenetic factors were present in those with the vascular symptoms, such non lipid hypothetical factors should also be carried by a good number of the asymptomatic group This may seem far fetched but should be considered

Of the 458 patients 23 had either intermittent claudication with positive arteriography or cerebrovascular lesions in the absence of cardiac symptoms These patients have been excluded from figures and tables with the exception of fig 6, illustrating triglyceride levels at the follow up examination and fig 3 analysing the total mortality Nine of these 23 developed myocardial infarction or angina pectoris during the time of observation and 9 died

The follow up study of the living included a detailed history of heredity diet, working ability eventual medication, a complete physical examination, blood pressure measurements, examination of palpable arteries, (and if necessary oscillometry) ECG, measurements of fasting levels of serum cholesterol and triglyceride In a minor part of the

material (40) a number of other investigations including work load on a *bicycle ergometer* was performed. Some of these findings will be discussed in a separate publication.

Chemical methods

Blood was sampled in the fasting state in the morning: cholesterol was determined according to the *Cramér* and Isaksson (4) modification of the Theorell method, triglyceride according to Carlson's (2) modification of the Carlson and Wadstrom (3) method. As this report covers a considerable period of time we will in the discussion come back to the question whether the serum cholesterol method has given a steady and unchanged level thoroughout this long time.

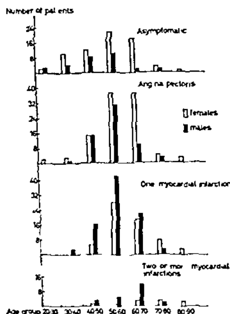


Fig. 1. Vascular symptoms in essential hypercholesterolemia in relation to age and sex at initial examination.

Results

Fig. 1 divides the material according to sex, *age and symptoms at the initial* examination. There is a majority of females in the asymptomatic as well as in the angina pectoris group, especially *striking above the age of 60. In the group* with one myocardial infarction males dominated up to the age of 60. Asymptomatic and angina pectoris males above the age of 60 were rare.

The small group of females who had an attack of myocardial infarction before the age of 50 showed very high cholesterol levels, *practically all of them above 400* mg % and none below 350 mg %.

Fig. 2 gives the percentage mortality at five years as well as during the whole observation time in the whole material arranged according to absence or presence and character of symptoms at the initial examination. The sex difference in prognosis seems apparent in the angina pectoris group and to some surprise still

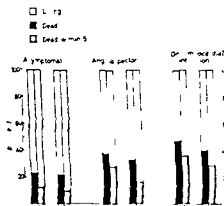


Fig. 2. Total and five year mortality in essential hypercholesterolemia in relation to sex and initial state of cardiovascular symptoms.

clearcut after one myocardial infarction.

While total mortality was 22 per cent in both sexes in the asymptomatic group

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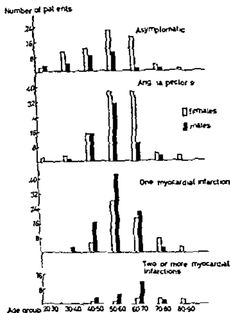


Fig 1 Vascular symptoms in essential hypercholesterolemia in relation to age and sex at initial examination.

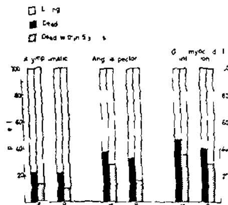


Fig 2 Total and five year mortality in essential hypercholesterolemia in relation to sex and initial state of cardiovascular symptoms

clearcut after one myocardial infarction

While total mortality was 22 per cent in both sexes in the asymptomatic group,

TABLE I Initial serum cholesterol level and mortality

	< 350 mg %				351—400 mg %				> 400 mg %			
	Strict diet		No or minor diet		Strict diet		No or minor diet		Strict diet		No or minor diet	
Sex years	Living	Dead	Living	Dead	Living	Dead	Living	Dead	Living	Dead	Living	Dead
Females												
< 40	2	—	5	—	—	—	1	—	3	1	4	—
41—50	4	1	12	2	3	—	8	2	4	1	3	1
51—60	13	—	20	9	6	—	16	13	6	3	5	2
61—70	7	1	18	15	5	—	7	11	2	—	4	4
71—80	—	1	3	8	—	—	2	3	—	—	—	4
81—90	—	—	—	3	—	—	—	—	—	—	—	—
Total no	27	3	58	37	14	—	34	29	15	5	16	11
Mean age	55	60	55	66	58	—	56	61	47	51	50	66
Mean observ months	84	91	85	45	86	—	87	49	88	94	95	54
Males												
< 40	4	—	5	1	—	—	1	2	1	—	1	—
41—50	13	2	14	9	1	—	2	12	2	1	4	2
51—60	10	1	19	24	7	3	7	4	1	—	5	2
61—70	5	1	10	18	2	1	2	—	1	—	2	2
71—80	1	—	—	5	—	—	—	—	—	—	—	1
Total no	33	4	48	57	10	4	12	18	5	1	12	7
Mean age	52	52	52	58	55	55	54	57	50	45	51	59
Mean observ months	80	57	82	41	76	61	75	56	92	41	86	29
Sum of both sexes	60	7	106	94	24	4	46	47	20	6	28	18
Percentage dead		10		47		14		51		23		39

the 5 year mortality in this group was 10 and 13 per cent, respectively and thus not big enough to give really dependable data They show the need for large materials and long observation times for the evaluation of measures directed towards derangements of serum lipid levels in asymptomatic cases

Level of hypercholesterolemia and prognosis

Tables I-III show the material divided according to sex, age at initial observation (arranged according to decades) and the influence of initial cholesterol levels on survival and mortality An idea is also given of how many patients adhered to a strict diet throughout their

TABLE II Changes of serum cholesterol in patients on strict diet and their controls 112 matched pairs

	Strict diet				Control				Change of serum cholesterol Mean percentage *reduction* Both individuals in matched pairs living		
	Initial no	Dead	Living	Follow up examin	Initial no	Dead	Living	Follow up examin	No	Strict diet	Control
										(%)	(%)
Females											
Asymptomatic	17	1	16	16	17	3	14	11	11	−19	−7
Angina pectoris	29	0	29	29	29	9	20	17	17	−18	−7
One myoc. inf	15	3	12	12	15	6	9	7	5	−27	−4
Total	61	4	57	57	61	18	43	35	33	−20	−6
Males											
Asymptomatic	8	0	8	8	8	0	8	6	6	−15	−19
Angina pectoris	22	6	16	16	22	8	14	11	10	−12	−20
One myoc inf	21	2	19	19	21	12	9	8	6	−7	−14
Total	51	8	43	43	51	20	31	25	22	−12	−18
Sum of both sexes	112	12	100	100	112	38	74	60			

TABLE III Weight at initial and follow up examinations (kg) in patients on strict diet and their controls

	Strict diet				Control			
	No	Initial	Follow up	Change	No	Initial	Follow up	Change
Females								
Asymptomatic	14	65 5	62 9	−2 6	10	67 1	66 2	−0 9
Angina pectoris	22	69 8	66	−3 8	11	73 4	74 6	+1 2
Total	36	68 1	65 2	−2 9	21	70 4	70 6	+0 2
Males								
Asymptomatic	5	75 8	78 5	+2 7	6	84 0	86 4	+2 4
Angina pectoris	10	74 2	75 0	+0.8	11	78 1	73.8	−4 3
Total	15	74 7	76	+1 3	17	80 2	78.2	−2

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41—50	4	1	12	2	3	—	8	2	4	1	3	1
51—60	13	—	20	9	6	—	16	13	6	3	5	2
61—70	7	1	18	15	5	—	7	11	2	—	4	4
71—80	—	1	3	8	—	—	2	3	—	—	—	4
81—90	—	—	—	3	—	—	—	—	—	—	—	—
Total no	27	3	58	37	14	—	34	29	15	5	16	11
Mean age	55	60	55	66	58	—	56	61	47	51	50	66
Mean observ months	84	91	85	45	86	—	87	49	88	94	95	54
Males												
< 40	4	—	5	1	—	—	1	2	1	—	1	—
41—50	13	2	14	9	1	—	2	12	2	1	4	2
51—60	10	1	19	24	7	3	7	4	1	—	5	2
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71—80	1	—	—	5	—	—	—	—	—	—	—	1
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the heavy dominance of cardiovascular deaths it should be pointed out that the vast majority of the whole series had their cholesterol measured originally either because *they themselves* had coronary symptoms or because of having siblings with coronary symptoms or because of the discovery of xanthomata or xanthelasmata

The effect of active attempts to lower the serum lipid levels

This is a particularly difficult question to assess, mostly because of the difficulty in delineating the group of patients who had such measures consistently applied from those who either for a short period submitted themselves to a rigid regimen, dietary or medicamental or both or who undertook some ill defined minor dietary restrictions throughout most of the years We have defined our group of actively treated as those where the records clearly show that they adhered to strict dietary control during at least 80 per cent of their total observation time and in a few where a continuous medicamental regimen was superimposed on the dietary regimen (thyroid analogues heparin, intravenously or subcutaneously or in one single case a combination of diet a high dose of estrogen and heparin twice weekly)

It was also required that these patients should have returned for regular controls including blood lipid measurements The diet up to the beginning of 1955 was a low fat low cholesterol diet After that the regular method of procedure was to put the patient for 1—2 months on a low fat low cholesterol diet and then to add food with a high content of polyunsaturated fatty acid, particularly edible oils or a highly unsaturated type of oleomargarine (Margo, Swedish Oleo margarine Company, Linoleic acid content 45 %) Attempts to calculate the consumption of edible oils have revealed that even those patients who began with eating 100 g of edible oil daily or more fairly soon settled down to a maximum daily dosage of about 50—60 g of oil This amount on the other hand seems to be well tolerated in practically all patients

The actively treated group numbers 121 patients A comparison of the mortality of this group with that of the rest of the material showed a very marked difference It was realized that on the whole the patients who had been picked out for and adhered to a strict regimen was a highly selected group These patients on the whole were younger, had higher serum lipid levels worse family history and more drastic symptoms There might also have been an elimination of patients with even mild cerebral deterioration, chronic ethylism or other factors which might play a general, unfavourable prognostic role

The rest of the material was either untreated or had had short lasting attempts on strict regimens or medicamental treatment or had such minor dietary changes as avoidance of excess butter and cream Out of this not actively treated material a matched control material was selected for each of the actively treated patients The match was made as to sex age blood pressure levels and degree of vascular symptoms observed and also as closely as possible to the cholesterol level at the start of the observation period The match was made

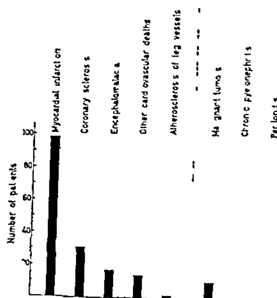


Fig 3 Causes of death in essential hypercholesterolemia myocardial infarction fully established diagnosis clinically and pathoanatomically Coronary sclerosis diagnosis by death certificate Encephalomalacia pathoanatomical diagnosis Other cardiovascular deaths include aortic stenosis, congestive failure, pulmonary embolism

course For definition of strict diet see below

It is seen that only few patients fell into the age groups beyond 70, particularly in the males, and also how very seldom these were put on and adhered to a strict diet

The average age at death in all groups was lower in the males Living males in higher age groups with high serum cholesterol were rare It is also seen that even in this large material of hypercholesterolemia, death at a very early age is a comparative rarity

There were very few patients in the higher age brackets in the group with cholesterol above 400 mg % Otherwise the mortality figures do not appear strikingly different from the other groups The patients with very high serum cholesterol levels were on the whole very well aware of their condition and adhered to their diet, even if they did not fulfil our criteria as belonging to the group of strict dieters It might also be pointed out that cholesterol levels above 400 mg % are rare in mixed hyperlipemia

The different age distribution among those adhering to a strict diet as compared with those with no, minor or inconsistent dietary changes prompted us to try to compare the strictly dieting group with as closely matched controls as possible (see below)

Fig 3 shows the causes of death The heavy dominance of cardiovascular deaths is clearly apparent — nearly 90 per cent of the deaths belonging to this group In the vast majority of autopsied cases it was possible to perform a rough grading of the state of the aorta and coronary vessels according to a scale from 0 to ++++ Four plus would for instance mean that the whole aorta, thoracic and abdominal, was more or less covered by ulcerating and calcified atheromatosis Atheromatosis in the pulmonary artery increased the grading as did the limited observations on atheromas in the femoral veins During earlier year these two regions were not investigated consistently Three plus was used to denote extensive ulcerative atheromatosis covering wide parts of the aorta In doubt the cases were referred to a lower grade In no instance in the entire series was a gradation made of 0 or one plus The cases dying of malignant tumors or chronic pyelonephritis and peritonitis on the whole showed lesions fully comparable with the rest of the series However, again when discussing

Table IV Initial average blood pressure in patients on strict diet and their matched controls divided into those who died and those who survived

	Survivors		Dead	
	Strict diet	*Control*	*Strict diet*	*Control*
Females	171/98 (n=58)	169/97 (n=43)	172/100 (n=3)	191/106 (n=18)
Males	145/93 (n=45)	144/93 (n=31)	153/99 (n=6)	161/96 (n=20)

Comparison of serum cholesterol levels at initial and follow up examinations

The average degree of control is seen in table II It is seen that the follow up was 100 per cent in the strictly treated groups In 55 instances both individuals in the matched pair lived and were examined While the average reduction of serum cholesterol in the group of strict diet seemed greater in the females — the opposite seemed true in the males

These findings should be compared with the results as regards weights For both individuals where weight was available the results have been put in table III

The cases with myocardial infarction have been omitted for obvious reasons as so many were first examined shortly after an acute episode From table III it appears that in both sexes the control material weighed somewhat more initially It also appears as if the surviving males in the control group had decreased their average weight — while the surviving strict dieters had gained This might be compared with the change in cholesterol levels in table II

We have also questioned whether one of the factors contributing to the great difference in mortality between strict dieters and controls might be explained as follows

Both series were matched closely including blood pressure However regularly controlled dieters might, if hypertensive have had their blood pressure controlled better than the controls, who mostly went out of our field of vision

As reliable data are lacking as regards blood pressure control in the dead control material, we have analysed this question as seen from table IV This table shows initial blood pressure as being slightly higher in those who died The difference between dieters and controls were not consistent

Among the surviving males, 3 strict dieters and one of the controls had antihypertensive treatment at the follow-up examination

The corresponding figures in the females were 22 (of whom only 5 had more potent drugs) versus 9 (2 of whom had drugs which were more potent) Although these figures in the females may be of some importance in explaining the higher survival rate, they cannot explain the difference in the male group The initial blood pressure figures

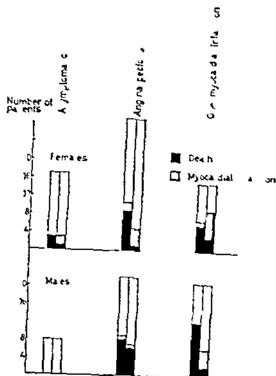


Fig 4 Recurrent myocardial infarction and death in essential hypercholesterolemia Right column (from the reader) patients on strict diet Left column patients matched as to age sex vascular symptoms blood pressure and cholesterol

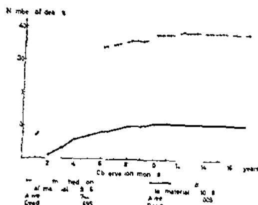


Fig 5 Cumulated deaths in patients on strict diet and matched controls Labelling as strict dieting has required at least three ambulatory visits First year mortality in matched controls might be excluded for reasons given in the text

by a secretary who had at the time no means of judging the course We were able to find close matches to 112 of 121 of the actively treated cases The five unmatched patients were dropped from the series, as were the cases with two or more myocardial infarctions

Fig 4 shows that in both sexes and in all the groups arranged according to symptoms there were fewer deaths in the actively treated groups (to the right) However, the total number of new myocardial infarctions (fatal or surviving) did not differ much The case records of the deaths were then worked through again in detail with the express purpose of establishing whether any case might have been placed in the wrong group as regards active treatment In fig 4 we have excluded the small number (4) of patients with two or more myocardial infarctions with their matched controls due to their heterogeneity For one patient with 4 myocardial infarctions who had been treated with diet, heparin and estrogen we could not obtain any adequate control whatsoever This patient has now for nearly 9 years been free from new attacks of myocardial infarction and his angina pectoris has disappeared completely This patient stands out from the rest of the observed series during these 16 years in these two departments

Fig 5 shows the cumulation of deaths in the actively treated patients and their matched controls The total number of observation months was definitely higher in the strictly treated series The difference seems clearcut However, it might be argued that the five patients in the control material dying during the first year had a very shortlasting opportunity to get on to a strict regimen and one might consider whether it would have been better to exclude these from the series

TABLE V The influence of diastolic blood pressure (mmHg) on mortality in *hypercholesterolemia*

	Males				Females			
	≤100		>100		<100		>100	
Diabetic B P	No	%	No	%	No	%	No	%
Asymptomatic								
Total no	24		6		52		14	
5 year mortality	2	(8)	2	(33)	3	(6)	4	(29)
Total mortality	4	(17)	3	(50)	9	17	6	(43)
Angina pectoris								
Total no	49		11		58		41	
5 year mortality	12	24	5	(45)	8	14	8	20
Total mortality	17	35	6	(55)	15	26	18	44
One myoc inf								
Total no	64		25		41		22	
5-year mortality	19	30	13	52	11	27	7	32
Total mortality	28	44	15	60	16	39	9	41
Sum								
Total no	137		42		151		77	
5-year mortality	33	24	20	48	22	14	19	25
Total mortality	49	36	24	57	30	20	33	43

two of these studies extensive comparisons were made between the method used and the Sperry and Webb procedure showing that the method used in this study at all cholesterol levels gave about 45 % higher values In various age groups the average serum cholesterol levels of healthy controls throughout this time corresponded fairly closely to results by other authors from populations in Sweden and other Western societies

The glyceride glycerol method was adopted without modification from Carlson (2) Our average values for both males and females correspond very closely to the figures given from Stockholm by this author (1)

The triglyceride method was not available at the start of the study At the follow up about 25 % of our patients had definite hyperglyceridemia and a further 20 % had borderline values This seems of some but limited interest The election due to death might have been considerable Presumably patients with hyperglyceridemia might have shown higher mortality There is also the very real risk that on being called to a follow up examination some individuals will tend to go on a temporary diet for a few days before the examination in order to

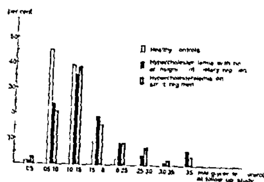


Fig 6 Fasting serum glyceride-glycerol at follow up study in a material of hypercholesterolemia The material was originally selected only on the basis of their cholesterol levels (2 or more serum cholesterol determinations > 300 mg %)

seem to speak in the direction of hypertension and differences in anti-hypertensive treatment only playing a minor part in the observed difference in mortality

Hypercholesterolemia and/or hyperglyceridemia

This material was collected up to the end of 1958 when we had no triglyceride method available We have considered it of interest at the follow-up examination to see how many of these patients selected only with regard to their cholesterol levels had elevated triglyceride levels

Fig 6 shows that in the material there were about 25 % who had clearly elevated triglyceride levels while about 20 % were in a borderline zone (Naturally, these figures do not allow of any conclusions about the frequency of hyperglyceridemia in the original material since there is a possibility that high triglyceride levels might have been over (or less likely under) represented in those who died before the follow up examination

Hypercholesterolemia and hypertension

The relationships between cholesterol, triglyceride and blood pressure will be treated in detail in a separate publication In this study we only aim to arrive at a rough idea of the influence on prognosis of definite increases of blood pressure

After placing those patients in a special group where the only available blood pressure measurements were made during the course of an acute myocardial infarction the rest of the material was arranged according to sex, degree of vascular symptoms and blood pressure at the initial examination Results are shown in table V The number of patients showing diastolic blood pressures above 120 and 140 mm Hg respectively were too few to allow of analysis A definite influence upon the mortality was clear in the group with a diastolic level above 100 mm Hg For the whole series including all subgroups with the exception of those observed only in the course of a fresh myocardial infarction the mortality of males with diastolic blood pressure above 100 mm Hg was 48 % as compared with 24 % for those below 100 mm Hg The corresponding figures for females were 25 % as compared with 14 %

Discussion

As this study covers a very considerable length of time — 17 years — it may be appropriate to ask whether the cholesterol method has remained constant throughout all these years Since 1955 we have made up new control materials from these laboratories (1, 4, 5 9) In

strict dieters are deducted from the 96 asymptomatic cases, the 71 remaining asymptomatic patients had 20 deaths

Summary

From the study of a material of 458 cases of essential hypercholesterolemia observed for 5—17 years the following main conclusions have been drawn

1 Vascular signs and symptoms appeared earlier in the male than in the female in hypercholesterolemia Asymptomatic males beyond 60 years were very few Females with myocardial infarction below 50 years were few and all presented very high levels of cholesterol
2 Five year mortality in the different groups (asymptomatic, angina pectoris one myocardial infarction) were higher in the males than in the females except in the small group with two or more myocardial infarctions
3 A diastolic blood pressure above 100 mm Hg contributed unfavourably to the prognosis in all subgroups
4 A group of 112 patients with continuous rigid regimen, *controlled regularly* showed a lower mortality in all subgroups than did a control group, matched according to sex, age, blood pressure degree of vascular symptoms and cholesterol levels In the control group there had been either none, minor or inconsistant dietary alterations The total rates of myocardial infarctions did not differ much in the two groups
5 Triglyceride (glyceride glycerol) levels at the follow up examinations were definitely elevated in 25 per cent of cases in the untreated' group, while 17 5 per cent showed figures in the high normal range
6 The series demonstrates the need for even larger materials and longer observation times in assessing the effect of factors influencing lipid levels on morbidity and mortality

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show favourable values This is a rather commonplace observation in our experience

The effect of a strict regimen

The present type of retrospective study is realized to have many pitfalls, no matter how sincerely the attempts of close unbiased matching have been performed To establish a case as rigidly controlled requires a certain period of observation One might possibly take care of this difficulty by eliminating the first year deaths from the control group There has also been a degree of selection in the attempts of establishing strict dietary control as patients of advanced age were being excluded On the other hand, rigid control was usually attempted in those with a strong family history, high lipid levels or marked symptoms

The decrease of serum cholesterol in the rigidly treated group was somewhat better in females, but in males somewhat worse than in the matched controls We are at a loss for an explanation of these findings Among various explanations suggesting themselves we might mention the following The spurious dieting might have been more effective than we have thought or the control material being called for an examination might have regarded this as a very special occasion and made a determined attempt at dieting just before the follow-up examination However this may be, it must be stated that the decrease of cholesterol in males on a strict diet was very moderate In this connection it may be mentioned that the patients adhering for such long periods to a diet might themselves be a selection of a very special kind, living cautious and prudent lives and thereby carrying less risk of mortality

However, all our experience has strengthened our opinion that whether or not a patient adheres to any type of therapy for such extended periods of time depends most of all of the relationships to the physician in charge and the attitude of this physician to chronic disease in general and to the therapeutic regimen used Owing to the lack of initial triglyceride determinations the degree of control of triglyceride can only be guessed at From wide experience during later years one might say that a reduction of about 15 % in total serum cholesterol is usually accompanied by about 25—30 % reduction in triglyceride levels As diet according to our experience produces a slight increase in α lipoprotein levels, the average reduction in the number and probably also the size of β lipoproteins might have been more pronounced than the changes in serum cholesterol levels indicate

In the group with myocardial infarction in the strictly dieting group only 2 of 22 males (of these none during the first 5 years) died as compared with 12 of 22 controls These figures seem strikingly low as compared with corresponding figures in most materials of myocardial infarction, collected with no regard as to the serum lipid levels However, the material is much too small for far reaching conclusions

At any rate 13 dead in the 112 strict dieters (asymptomatic, angina pectoris and patients with one myocardial infarction) compares favourably with 21 dead in the group of 96 asymptomatic cases, taken as a whole Furthermore, if the 25

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Blood Glucose, Free Fatty Acids and Intravenous Glucose Tolerance Test in Obese Patients on Different Diets

By

PER BJORNTORP ANDERS JONSSON and BERTIL HOOD

The dependence of the glucose tolerance test on the diet preceding it is well established A delayed disappearance has been noted after a low carbohydrate diet, with a return to normal after a period on a diet adequate in calories and high in carbohydrate (1 5 7 9 10 15 16) Excess carbohydrate causes no further change (5)

An abnormally slow rate of disappearance of glucose loads in obesity has been observed by several authors (1 2 14, 18) Stable obesity has been thought especially to pre dispose to this abnormality (13 14) Diabetic type glucose tolerance curves have been observed in the absence of glucosuria and with a normal fasting blood sugar (14) The diet before the tests does not seem to have been controlled in all of these studies

Dole (7) observed abnormally high fasting levels of free fatty acids in venous blood in obesity This finding has later been repeatedly confirmed (3 6 12)

In the present investigation blood glucose free fatty acids and glucose tolerance have been determined in obese patients while dietary conditions have been varied and defined

Material

Seventeen patients with pronounced obesity weighing between 82 6 and 140 1 (average 110 2) kg 14 women and 3 men ages 18 — 61 years were included All were more than 35 % above desirable weight (11) None suffered from infections during investigation, and none of the patients had glucosuria or were on drug treatment Most of the patients had gall-stones and a previous history of gall bladder disease but none had laboratory evidence of biliary obstruction

All patients were hospitalized during investigation On admission 7 patients were given an ordinary 2 500-calorie diet with the addition of carbohydrate meals between ordinary meals for at least three days before investigation They were then given a 500-calorie diet and investigations were again performed after at least a week The other 10 patients were directly given the 500-calorie diet and were not examined on the higer calorie diet.

Eight medical students or patients clinically free from metabolic disorders weighing

Submitted for publication February 9 1965



differ significantly ($p > 0.05$) either between groups or between the obese on different diets K values were in all cases above 1 10 in the normals In the obese on the 2 500 calorie diet with in between meals the average was lower than the normal value although not significantly different statistically Four had a k value below 0 90 On the calorie restricted diet a still lower average k value was found although not statistically significantly lower than that for the obese on a higher calorie diet In all cases, however, the k value fell after changing from a high to a low calorie diet The lowest value noted was 0 56

In table II the results of free fatty acid determinations are listed Normals increased their venous blood level significantly ($p < 0.02$) between the 11th and the *20th hour* of fasting The obese patients on a calorie restricted diet had a significantly higher value at 11 hours of fasting than the normals ($p < 0.01$) This value did not increase over the following periods ($p > 0.05$) and was at 20th hour of the fast not significantly different from the corresponding value for the normals In the 8 obese patients who were on the higher calorie diet, comparisons were performed only between their values on the two diets in question On the 2 500-calorie diet with carbohydrate additions the obese patients had a significantly lower 11 hour value ($p < 0.02$) than on the low calorie diet but not significantly different from the corresponding value in the normal group The 20-hour fasting value in the obese on the higher calorie diet was significantly higher than the 11 hour value for the same group ($p < 0.05$), but not

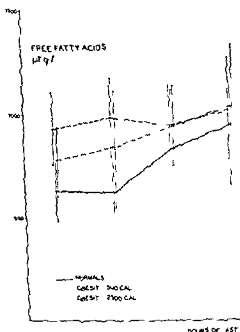


Fig 1 Free fatty acid values (averages and standard deviations) after different times of fasting for normals and obese subjects on different diets.

significantly different from the corresponding value in the normal group or the obese group on calorie restricted diet The average values of free fatty acids for the obese on the higher calorie diet were, however higher than the normals at each time measured When compared on a relative basis the mean of all the observations on the obese patients in the group in question is significantly higher ($p < 0.01$) than the mean for the normals These results are also shown graphically in fig 1

Discussion

A diabetic type glucose tolerance test after a period of low caloric intake has been observed repeatedly (1, 5 7 9 10

TABLE I Fasting blood sugar values and intravenous glucose tolerance k values for obese patients and normals Mean ± S D Within parentheses are values for the patients on the diet containing 500 cal, who were also investigated on the 2,500 cal diet

	Obesity		
	2,500 cal diet	500 cal diet	Normals
Fasting blood sugar (mg per 100 ml blood)	97±7 n=8	89±12 (90±9) n=16 (n=8)	77±15 n=7
K values[1]	0 95±0 23 n=8	0 84±0 24 (0 76±0 21) n=10 (n=8)	1 40±0 20 n=7

[1] Rate of glucose disappearance, according to Amatuzio et al (1)

TABLE II Serum free fatty acids (μEq/l) after different fasting periods, for obese patients and normals Mean ± S D Within parentheses are values for the patients on the diet containing 500 cal, who were also investigated on the 2,500 cal diet

	Obesity		
Hours fasting	2,500 cal diet	500 cal diet	Normals
11	784±183 n=8	926±175 (982±225) n−17 (n=8)	631±150 n=8
14	832±278 n=8	968±191 (1,030±212) n=17 (n=8)	618±267 n=8
17	938±197 n=8	941±204 (938±207) n=17 (n=8)	794±314 n=8
20	1,016±298 n=8	1,009±297 (1 122±120) n=17 (N=8)	906±261 n=8

61 9 to 74 8 (average 66 0) kg 5 men and 3 women ages 22—45 years, served as a healthy control group

Methods

The intravenous glucose tolerance test was performed as described by Goldberg and Luft (5) Glucose was analyzed enzymatically in capillary blood (8)

Estimation of free fatty acids during prolonged fasting was performed at 11, 14, 17 and 20 hours after the start of fasting During this period the patients were allowed to walk and to drink water but not to smoke or perform heavy work Free fatty acids were determined in serum from blood drawn from the antecubital vein (4)

Results

Table I gives the fasting blood sugar values and the intravenous glucose tolerance test k values for the both groups The fasting blood-sugar value did not

their 11 hour fasting free fatty acid level, which now no longer was significantly different from the normals After 20 hours of fasting no significant differences were found between groups or between the obese on different diets However, all average values of free fatty acids taken at 11, 14, 17 and 20 hours of fasting were higher in the group of obese patients on the higher calorie diet than the corresponding normal values When all values were compared on a relative basis the obese patients values were significantly higher The importance of defining the diet in studies of metabolism in obesity is emphasized

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15, 16) This corresponds to a sluggish response of the plasma-insulin concentration to oral glucose as measured by an immunological technique (17) An insensitivity of adipose tissue to insulin, primarily affecting the free fatty acid release mechanisms, has also been suggested (7) The same observation now made in obese patients on calorie restricted diet might be a similar phenomenon

On a diet adequte in calories and with addition of carbohydrates, the glucose-tolerance test in the obese patients on an average approached normal This is probably only true for obesity when patients with glucosuria and other signs of clinically overt diabetes mellitus have been excluded

Free fatty acids in the group of obese patients on a calorie restricted diet were high after 11 hours of fasting and did not increase during prolonged fasting They were, however, not statistically different from the normal values after increasing calorie intake The early increased levels on a low-calorie diet might correspond to the demonstrated impairment of glucose disappearance A decreased glucose uptake in different tissues would account for the low k-values of the glucose tolerance tests, and an impairment of adipose tissue uptake of glucose could be thought to cause the higher levels of free fatty acids earlier during fasting Glucose uptake in adipose tissue seems necessary for reesterification of fatty acids to triglycerides (18) When a decrease in adipose tissue glucose uptake thus occurs, an increased outflow of free fatty acids will result and hence also an increase in plasma free fatty acids

The phenomenon of a high level of free fatty acids after about 12 hours of fasting and a very slow or no increase at all for the next 10 hours has earlier been described in obese patients (3, 6) The results reported in the present work emphasize the importance of defining diet before conclusions can be drawn about a possible derangement of free fatty acid metabolism in obesity

On an average the values for free fatty acids in obesity after a full diet were higher than in the normals Whether this means an abnormality of free fatty acid metabolism in established obesity or is just a result of a larger number of fat cells, contributing to the plasma pool of free fatty acids, cannot be decided at present

Summary

Blood glucose, free fatty acids and intravenous glucose tolerance was determined in obese patients on diets containing either 500 calories or 2,500 calories with carbohydrate additions, and compared with the values for normals No patients with glucosuria were included Fasting blood sugar showed no differences Intravenous glucose disappearance showed no statistical differences but was in each case of obesity changed to a slower disappearance rate when the change of diet from higher calories to calorie restriction was introduced After 11 hours of fasting the free fatty acids of the obese on a calorie-restricted diet were significantly higher than corresponding values for the normals on an *ad libitum* diet When given the higher calorie diet with carbohydrate additions, the obese significantly decreased

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Serum Lipids and Glucose Tolerance in Subjects with a Family History of Diabetes Mellitus

By

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In recent years a great number of investigations have been published concerning alterations of blood lipids in non diabetic as well as in diabetic individuals and the relationship of these findings to the development of atherosclerosis has been widely discussed Alterations of serum lipids are of particular interest in diabetic patients in whom an increased incidence of atherosclerotic and other forms of vascular lesions are known to exist

It is well known that in uncontrolled diabetics the inability to handle glucose at a normal rate has a pronounced effect on fat metabolism and can result in an overproduction of ketone bodies and the development of ketosis This is accompanied by marked alterations in the amount of circulating blood lipids (17) More recently it has been established however that abnormalities of blood lipids may exist in diabetic patients even in the absence of ketoacidosis or renal disease According to Adlersberg and Eisler these abnormalities consist mainly of an elevation of serum triglycerides and free fatty acids (NEFA) with only moderate or no increase in serum cholesterol and phospholipid levels (2)

Increased serum triglyceride levels in well controlled juvenile diabetics have also been observed by Albrink and Man (4) although this lipid fraction according to more recent observations is only higher in diabetics over the age of thirty as compared with non diabetics (24) Plasma cholesterol levels, on the other hand, have been found to be higher than in non diabetics throughout the life span while neither the triglyceride concentration nor the plasma cholesterol level has been found to be related to the known duration of diabetes (24) It is furthermore noteworthy that although serum triglycerides and NEFA are usually the only lipid fractions which are significantly increased in well-controlled juvenile diabetics there may be an increase in all lipid fractions in elderly controlled diabetics a type of hyperlipidemia which is frequently found in non diabetic patients with atherosclerosis (29)

Submitted for publication February 11 1965

istering to the test subjects 1 g of glucose per kg of body weight Venous blood was withdrawn for the blood glucose determinations at 0 1/2 1 1 1/2 2 (2 1/2) and 3 hours after the glucose load A method for "true" glucose determination was used (18)

Prior to the administration of the glucose load blood samples were withdrawn for the following lipid determinations Serum triglycerides were determined by a slightly modified procedure of Van Handel and Zilversmit (33) serum cholesterol was determined by the method of Pearson et al (25) and plasma NEFA levels were analyzed by the Dole procedure as modified by Trout et al (32) In addition the electrophoretic pattern of the serum lipoproteins was studied by means of paper electrophoresis Blood samples for the determination of NEFA were in addition obtained one hour and three hours after the administration of the glucose load simultaneously with the corresponding blood glucose samples for the PGTT

An additional control material consisting of 13 healthy individuals without a family history of diabetes was examined in a similar way The age distribution of this control material was similar to that of the relatives of diabetic patients

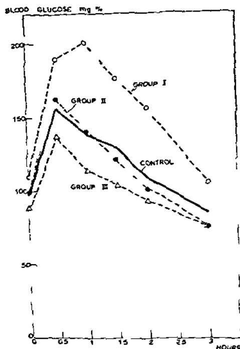


Fig 1 Prednisone glucose tolerance curves in relatives of known diabetics and in control subjects without known diabetic relatives The glucose tolerance curves obtained in the subjects with a diabetic family history are divided into three groups according to criteria explained in the text.

Results

Glucose metabolism

The results of the prednisone glucose tolerance tests (PGTT) were interpreted according to the criteria set forth by Fajans and Conn for their cortisone glucose tolerance test (8 13) Relatives of diabetic subjects who had a one hour blood glucose value above 160 mg% and a 2 hour blood glucose value above 140 mg% were thus considered to have a "positive response" and these individuals were designated as group I (table 1) Additional subjects examined during the course of the investigation had peak blood glucose values above 160 mg% often accompanied by glycosuria but the blood glucose curve approached normal values more rapidly than in the previous group and did thus not meet the criteria for a "positive response" according to Fajans and Conn These individuals were designated as group II The last group (group III) consists of relatives of diabetic subjects whose blood glucose values during the PGTT did not exceed 160 mg% at any point and who did not show any glycosuria during the test It should be noted that two additional subjects with a family history of diabetes and without any previous known disturbance of carbohydrate metabolism

TABLE I Results of prednisone glucose tolerance tests performed in 60 subjects with a family history of diabetes

Group I	Group II	Group III	Group IV
1 hr BG[1] > 160 mg% and 2 hr BG[1] > 140 mg %	Peak BG[1] value > 160 mg % and/or glycosuria	Peak BG[1] value < 160 mg % No glycosuria	Manifest diabetes
13 (21 7 %)	20 (33 3 %)	25 (41 7 %)	2 (3 3 %)

[1]BG = blood glucose

The existence of alterations in the circulating serum lipids of diabetic patients which are not due to insufficient control of the diabetic state implies that the hyperlipidemia of these diabetic subjects might be due to a basic metabolic fault which possibly could be demonstrated already at a very early stage of the disease simultaneously with or even before the occurrence of disturbances of the glucose metabolism Recently increased attention has been focused on the so-called asymptomatic or preclinical stage of diabetes which can be demonstrated only by the use of a cortisone glucose tolerance test or other similar procedures (8) and it has been shown that vascular lesions as well as diabetic nephropathy or neuropathy can be present already in patients who exhibit only a minimal derangement of carbohydrate metabolism (10, 11, 12, 15) Since there seems to be reason to believe that additional information on the nature of the alteration of serum lipids in diabetic patients could be obtained by studying such patients at a very early stage of their disease determinations of various serum lipids were carried out in a group of relatives of known diabetics, and an attempt was made to subdivide this group into asymptomatic diabetics and persons without demonstrable disturbances of carbohydrate metabolism according to the results of prednisone glucose tolerance tests which were performed in these individuals

Material and methods

The material consists of 60 close relatives (parents children or siblings) of known diabetics who were not previously known to have any disturbances of carbohydrate metabolism The diabetic relatives, who were treated at the diabetic out patient department of the Maria Hospital in Helsinki, consisted mainly of juvenile onset diabetics with a mean age of 43 years and an average insulin requirement of 56 units daily Five cases of mild diabetes not requiring any insulin treatment were also included

The experimental subjects underwent a complete physical examination including routine laboratory tests and were instructed to consume a diet rich in carbohydrate for three days prior to the prednisone glucose tolerance test (PGTT) The test was performed according to the original cortisone-glucose tolerance test described by Fajans and Conn (13) substituting 10 mg of prednisone for each 50 mg of cortisone Persons weighing less than 72 5 kg (160 lb) received thus 10 mg of prednisone and persons weighing more than 72 5 kg received 12 5 mg of prednisone 8 1/2 hours and 2 hours before a standard glucose tolerance test which was performed by admin-

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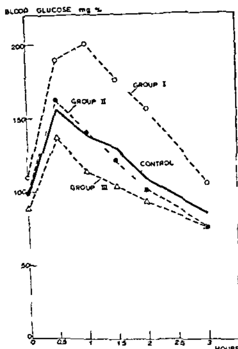


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to group I than in the relatives of the subjects belonging to groups II or III in which the duration of diabetes was 9 7 years and 11 6 years respectively but the difference was not found to be statistically significant

Clinical signs in the three groups of subjects with a family history of diabetes are listed in table III It should be noted that most cases of obesity or hypertension belonged to groups I or II while persons belonging to group III showed no cardiovascular derangement and only three persons belonging to this group were obese Persons with excessive birthweight or women with overweight children belonged likewise predominantly to groups I and II

In order to test to what extent the administration of prednisone contributed to the observed decrease of glucose tolerance in the individuals with a family history of diabetes ordinary glucose tolerance tests without priming with prednisone were carried out in 10 subjects who showed a marked impairment of glucose tolerance during the PGTT The glucose tolerance tests were carried out on an average 4 months (2 to 7 months) after the prednisone glucose tolerance test except in one case where the glucose tolerance test preceded the PGTT The glucose tolerance tests were except for the omission of the two prednisone doses carried out in the same way as the prednisone glucose tolerance tests The average prednisone glucose tolerance curves and ordinary glucose tolerance curves obtained in the same individuals are shown in fig 2 The blood glucose values during the prednisone test were markedly more elevated than the values observed after an ordinary glucose load and the difference between the two groups was statistically significant (p 0 01) It can be seen that the average blood glucose curve obtained during the standard glucose tolerance test is within the normal range These individuals did thus not have diabetes although they can be classified as asymptomatic or preclinical diabetics

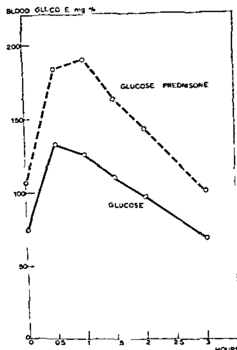


Fig 2 Comparison of the mean prednisone glucose tolerance curves and standard glucose tolerance curves obtained in 10 subjects with a family history of diabetes

Serum lipids

The average serum lipid values of non diabetic relatives of known diabetics and those of control subjects without a family history of diabetes are shown in table IV It can be seen that the mean values of the serum cholesterol triglycerides and

TABLE II Relationship of subjects with a diabetic family history to known diabetics

	Group I (13)		Group II (20)		Group III (25)		All groups (58)
Parents							
One	5	6 (26 1%)	10	11 (47 8%)	6	6 (26 1%)	23 (100%)
Both	1		1		0		
Children	4	(33 3%)	4	4 (33 3%)	4	(33 3%)	12 (100%)
Siblings	3	(13 1%)	5	5 (21 7%)	15	(65 2 %)	23 (100%)

TABLE III Clinical symptoms and signs in subjects with a diabetic family history

	Group I (13)	Group II (20)	Group III (25)	All groups (58)
Obesity[1]	3	6	3	12
Hypertension	1	6	0	7
Angina pectoris	2	0	0	2
Excessive birthweight (> 4 000 g)	2	1	0	3
Overweight children (> 4 000 g)	2	1	1	4

[1] Increase of weight > 20 % of ideal weight

were found to have a manifest diabetes with marked glycosuria

The average prednisone glucose tolerance curves of the above mentioned three groups of relatives of diabetic subjects and that of a control group without a family history of diabetes are shown in fig 1 It can be seen that the blood glucose curve of the relatives of diabetic patients belonging to group I was markedly more elevated than in the control group whereas group II was not significantly different from the control group and the blood glucose curve in group III was even less elevated than in the normal controls

The relationship to known diabetics in the above mentioned subjects is indicated in table II For every subject with a family history of diabetes the closest relative who was known to have diabetes was chosen and if a person, e g, was known to have both a diabetic parent and diabetic siblings only the parent was taken into consideration It can be seen that the majority of individuals who had diabetic parents had some abnormality of carbohydrate tolerance and belonged either to group I or group II while most subjects who had diabetic siblings did not reveal any abnormality when tested by the PGTT Subjects who had diabetic children were equally distributed between the three groups

There was no significant difference between the age or the insulin requirement of the diabetic relatives of the experimental subjects belonging to the three different groups The average duration of diabetes was found to be somewhat longer (15 3 years) in the diabetic relatives of the subjects belonging

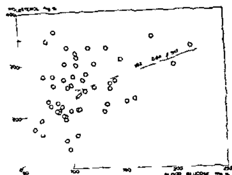


Fig 3 Correlation between fasting serum cholesterol levels and 2 hour blood glucose levels observed in relatives of diabetic patients during a prednisone glucose tolerance test

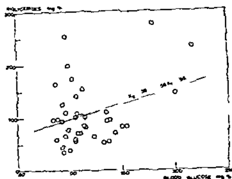


Fig 4 Correlation between fasting serum triglyceride levels and 2 hour blood glucose levels observed in relatives of diabetic patients during a prednisone glucose tolerance test.

lation coefficient of Spearman for the cholesterol values and the blood glucose values was $r = 0.38$ This is a statistically significant correlation ($p < 0.01$) The correlation coefficient obtained for the serum triglyceride values and the blood glucose values was $r = 0.32$ and the correlation between these two values was not statistically significant It can be seen that despite the positive correlation which was found to exist between the serum cholesterol level on the one hand and the 2 hour blood glucose values on the other, there were many individual cases where such a correlation could not be seen

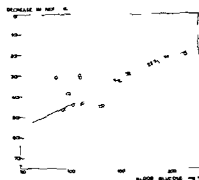


Fig 5 Correlation between the decrease of NEFA levels and the 2 hour blood glucose levels observed in relatives of diabetic patients during a prednisone glucose tolerance test The decrease of NEFA values is expressed as a percentage of the fasting level.

The decrease of the NEFA values during the PGTT one hour after the administration of the glucose load is in fig 5 plotted against the 2 hour blood glucose values obtained during the same test The alterations of NEFA levels following the oral administration of glucose are expressed as a percentage decrease of the initial fasting NEFA levels compared with the NEFA levels which were measured one hour after the administration of glucose The correlation coefficient of Pearson for the decrease of NEFA values and the 2 hour blood glucose values was $r = 0.46$ and a statistically significant correlation ($p < 0.01$) could thus be established between these two groups The correlation between the NEFA levels which were measured three hours after the administration of the

TABLE IV Serum lipids in three groups of relatives of diabetic patients and in control subjects without a diabetic family history

	Cholesterol (mg %)	NEFA Before PGTT 0 value (μEq/l)	NEFA After PGTT 1 hr Percent of 0 value	NEFA After PGTT 3 hrs Percent of 0 value	Triglycerides (mg %)
Control group					
N	13	13	12	13	13
$\bar{x}$	251	720	68 8	79 9	84 0
s	52 4	194	14 1	33 3	45 5
ε	14 54	54	4 07	9 24	12 62
Group I					
N	13	10	10	9	10
$\bar{x}$	282	801	64 0	64 6	121 7
s	56 24	229	16 41	36 39	74 96
ε	15 60	72 5	5 19	12 13	23 70
Group II					
N	18	16	16	16	16
$\bar{x}$	245	776	54 8	66 8	99 4
s	49 8	173	16 6	26 1	44 5
ε	11 74	43	4 15	1 52	11 12
Group III					
N	24	13	13	12	12
$\bar{x}$	247	751	60 2	71 8	103
s	59 1	193	12 4	25 7	61 4
ε	12 06	53	3 44	7 42	17 73

NEFA were somewhat higher in the subjects with a family history of diabetes belonging to group I than in the subjects who showed less impairment of the glucose tolerance or in the control subjects A statistical comparison of the serum lipid values in the different groups by means of the Wilcoxon test failed, however, to establish any statistically significant differences

In addition to the serum lipid determinations shown in table IV the electrophoretic pattern of the serum lipoproteins of the same subjects was studied by means of paper electrophoresis but no difference between the α, β or γ fractions could be noted in the different groups

The combined serum cholesterol and triglyceride values obtained in the relatives of diabetic patients are in figs 3 and 4, respectively, plotted against the 2 hour blood glucose values obtained in the same individuals during the PGTT The corre-

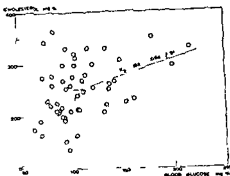


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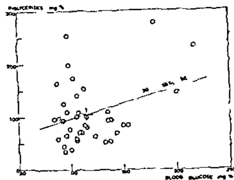


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Fig 5 Correlation between the decrease of NEFA levels and the 2 hour blood glucose levels observed in relatives of diabetic patients during a prednisone glucose tolerance test. The decrease of NEFA values is expressed as a percentage of the fasting level

The decrease of the NEFA values during the PGTT one hour after the administration of the glucose load *is in* fig 5 plotted against the 2 hour blood glucose values obtained during the same test The alterations of NEFA levels following the oral administration of glucose are expressed as a percentage decrease of the initial fasting NEFA levels compared with the NEFA levels which were measured one hour after the ad*ministration of glucose* The *correlation* coefficient of Pearson for the decrease of NEFA values and the 2 hour blood glucose values was $r = 0.46$ and a statistically significant correlation ($p < 0.01$) could thus be established between these two groups The correlation between the NEFA levels which were measured three hours after the administration of the

glucose load and the 2-hour blood glucose levels was on the other hand not statistically significant

Discussion

The number of "positive" prednisone-glucose tolerance tests (21 7 %) was somewhat smaller than the number of the subjects showing a positive response (26 %) in the cortisone glucose tolerance tests carried out by Conn and Fajans (8) in a more extensive study of non-diabetic relatives of persons known to have diabetes

Although the tests were carried out in the same manner and equivalent amounts of prednisone were substituted for cortisone which was used for priming in the original test of Conn and Fajans, it is still possible that the diabetogenic effect of the two steroids is not the same and, on the other hand, the glucose load which was used in the present study was somewhat smaller than that used by the above mentioned investigators

Additional factors which influence the interpretation of steroid glucose tolerance curves are the occurrence of positive responses in non-diabetic individuals without a family history of diabetes and the influence of age Three positive" responses were thus obtained in 13 healthy individuals without a family history of diabetes This high figure is somewhat disconcerting but although the lack of a family history of diabetes does not exclude the possibility that these individuals would actually belong to the group of diabetes suspects there was otherwise no valid reason for excluding them from the control material

The decrease of glucose tolerance with increasing age has been demonstrated by several investigators (8, 19, 20) and it has been suggested that different criteria for the interpretation of a glucose tolerance test should be adopted for persons over the age of 45 (28) It can, however, also be postulated that the diabetic trend is much more common among older non diabetic relatives of diabetic patients than heretofore believed and that a large number of such persons are able to resist successfully the development of diabetes although the ability of the β cells to respond to further stimulation is limited (8) The average age of group I of the persons with a diabetic family history included in the present study was 46 years and the average age of the control group which had a similar age distribution was 45 years Although the average age of the persons belonging to both groups II and III of the material was 37 years and there was a certain tendency towards elevated blood glucose curves in older individuals belonging to these groups it was, nevertheless, decided to use similar criteria for the interpretation of the prednisone-glucose tolerance curves irrespective of age

The finding of 12 cases of marked obesity (20 6 %) and 7 cases of hypertension (12 %) among the examined presumably healthy relatives of known diabetics is noteworthy and would seem to indicate an increased incidence in diabetes suspects of these conditions which are known to be commonly associated with manifest diabetes It is moreover of interest that most cases of obesity or hypertension belonged to the groups which showed either a positive response"

in the PGTT or at least some derangement of carbohydrate tolerance

Alterations of serum lipids *in the preclinical stage* of diabetes mellitus are of interest because of the association of such alterations and vascular lesions during the later stage of the disease. Although a positive correlation between these findings does not necessarily prove the existence of a causal relationship many investigators in the field hold the view recently expressed, e g by Albrink (3) according to which an excess of certain circulating lipids in addition to injury or genetically determined weakness of the arterial wall and intravascular clotting phenomena play an important role in the development of atherosclerosis

An association between serum cholesterol and atherosclerosis in diabetic patients has generally not been found to exist and it has been pointed out that an excess of serum cholesterol or other circulating lipids cannot be interpreted as a predictive sign of diabetic vascular complications in any one individual (22). The significance of the observed correlation between serum cholesterol and impairment of glucose tolerance in relatives of diabetic patients must be viewed against this background and it should furthermore be noted that the cholesterol values of the individuals who showed most impairment of glucose tolerance during the PGTT were not significantly higher than in the other subjects examined during the course of the present investigation

A certain tendency for the serum triglycerides to be elevated was observed in some subjects during the present study with a diabetic family history who at the same time exhibited a marked impairment of glucose tolerance after the administration of a glucose load. Although this finding lacked statistical significance it would seem to be in accordance with previous observations according to which serum triglyceride levels are increased in non diabetic relatives of diabetic patients (5). These observations might be important in view of the suggested association between elevated triglyceride levels and atherosclerotic involvement in diabetic patients (2, 6) although direct evidence for a connection between these findings is still lacking

The tendency towards increased levels of NEFA which was also observed in individuals with a family history of diabetes is in accordance with similar findings in uncontrolled as well as in well regulated manifest diabetics (7, 29). It has been assumed that the increased levels of NEFA reflect an impaired ability to limit the output of fatty acids from tissue stores and since the entry of glucose into tissue cells is known to be impaired in diabetes it has been suggested that insufficient α glycerophosphate which is derived from glucose was available for the normal esterification of free fatty acids resulting in increased levels of NEFA in the plasma (21)

The basic abnormality would thus consist of a deficiency of biologically active insulin either due to a diminished secretion of insulin from the pancreatic β-cells or to an excessive destruction or binding of circulating insulin

Randle et al have advanced a different point of view (16, 26). They have produced evidence suggesting that the primary event in the development of diabetes

might be an abnormality of glyceride metabolism which leads to an increased release of fatty acids from adipose tissue The result would be resistance to the hypoglycemic action of insulin followed by a rise in fasting glucose and insulin with eventual exhaustion of the pancreatic β-cells Their conclusions have been supported by the finding of increased lipolytic activity in the plasma of diabetic patients (27) and by the recent demonstration that noradrenaline induced rise in NEFA results in an impairment of the removal of intravenously administered glucose (23)

The rate of fall of plasma concentrations of NEFA following an oral glucose load has also been studied by Hales and Randle (16) and found to be delayed in diabetics or in people on a low-carbohydrate diet This was thought to be consistent with a delayed insulin action caused by the increased release of fatty acids for oxidation and consequent inhibition of glucose uptake in the tissues It is possible that the delayed decrease of NEFA levels observed during the course of the present study in those subjects who showed a marked elevation of blood glucose values following an oral glucose load could be explained along the same lines

Whatever the causal relationship may be between plasma concentrations of NEFA and other lipid fractions on the one hand and a decrease of glucose tolerance on the other the results of the present investigation are suggestive of alterations of serum lipids in individuals with a family history of diabetes which at the same time show an impairment of glucose tolerance during the PGTT These individuals are in all probability potential diabetics and alterations of blood lipids in these subjects possibly indicate an increased disposition to the development of atherosclerotic vascular disease or other vascular manifestations which are known to be associated with diabetes The practical importance of detecting abnormalities of circulating lipids in these early diabetics would then lie in the possibility that the alterations in lipid metabolism might still be reversible at this stage by appropriate dietary measures in combination with drugs which improve carbohydrate utilization and affect lipid metabolism Reports on the beneficial effects of tolbutamide (9, 14) or chlorpropamide (31) in improving carbohydrate tolerance during the asymptomatic or preclinical stage of diabetes have been published and attention should also be drawn to the observations that sulphonylurea drugs are capable of reducing hyperlipemia and hypercholesterolemia in patients with only minimal impairment of glucose tolerance (30)

It can be concluded that alterations of serum lipids during the early stages of diabetes would merit further study Long-term studies in diabetes suspects with concomitant observations of serum lipid levels and carbohydrate tolerance would seem to be indicated and such studies would in all probability throw further light on the significance and causal interrelationship of the metabolic alterations observed in subjects with a diabetic family history

Summary

Prednisone glucose tolerance tests (PGTT) were carried out in 60 non dia

betic relatives of known diabetics A positive" response according to the criteria used by Fajans and Conn was obtained in 13 individuals (21 7 %) while 20 additional relatives of known diabetics (33 3 %) showed only slight impairment of glucose tolerance and 25 per sons (41 7 %) had no demonstrable abnormality of glucose tolerance

Twelve persons included in the present investigations were markedly obese (20 6 %) and 7 had hypertension (12 %) The subjects who showed an impairment of glucose tolerance during the PGTT had frequently diabetic parents while the majority of the persons who had diabetic siblings did not display any derangement of glucose tolerance

Determinations of serum cholesterol, triglycerides and non esterified fatty acids (NEFA) were carried out in the relatives of diabetic patients and in a control group consisting of healthy subjects without known diabetic relatives and in addition an electrophoretic analysis of serum lipoproteins was carried out The average values of the serum cholesterol triglycerides and NEFA were found to be somewhat more elevated in the group of subjects who showed most impairment of glucose tolerance during the PGTT than in the group with a negative response or in the control subjects These findings were, however not statistically significant A statistically significant correlation was on the other hand found to exist between the combined serum cholesterol values of the subjects with a diabetic family history and the 2 hour blood glucose values observed in the same individuals during the PGTT A significant correlation was likewise observed between the decrease in NEFA one hour following the administration of the glucose load during the PGTT and the 2 hour blood glucose values determined during the same test

It is concluded that in some relatives of known diabetics significant alterations of circulating blood lipids can be demonstrated and that these alterations tend to be more pronounced in the individuals who show definite impairment of glucose tolerance as measured with the PGTT The significance of these findings is discussed

Acknowledgement

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From the Department of Medicine IV, Sodersjukhuset, Stockholm, Sweden

Calcium Studies in Partially Gastrectomized Patients, with Special Reference to the Oral Intake of Calcium

By

K Ekbom and R Hed

As early as 1939 Ask Upmark predicted that partial gastrectomy might give rise to osteomalacia (2) It is, however, only in recent years that any great interest has become focused on disturbances in calcium metabolism following gastric surgery

Nicolaysen and Ragård found a negative calcium balance in some gastrectomized patients (21) Pyrah and Smith gave an account of a 52 year-old woman in whom severe osteomalacia developed 14 years after partial gastrectomy (23) A number of other cases of osteomalacia as a sequela of gastrectomy have been reported (3 4 10 14 15 19)

Harvald et al found a pathological calcium metabolism in almost half of 38 cases studied (15) and later reports have confirmed the common occurrence of a disturbance in calcium metabolism after gastrectomy As a rule the cause has been presumed to be steatorrhoea with a loss of calcium in the faeces Gastrectomized patients do however often have a poor appetite and we have observed that they seldom tolerate milk and cheese, which are the main dietary sources of calcium Since the role of a low calcium intake has been little discussed in the literature, we have studied the effect of the calcium intake on the calcium metabolism after partial gastrectomy

A calcium infusion test according to Nordin and Fraser (22) was made in 15 patients The spontaneous dietary intake of calcium was also determined, and related to that in a control group of patients who had not undergone gastrectomy

Material and methods

In a follow up study of 317 patients who had undergone partial gastrectomy at Sodersjukhuset in 1955—1957 (8) we selected those who had a history of symptoms of malabsorption i e anaemia weight loss and diarrhoea with or without postcibal symptoms altogether about 35 patients Of these 14 consecutive cases were hospitalized for more detailed investigation To this material we added one patient (case 2) who had undergone partial gastrectomy by the Billroth II method at our hospital in 1947 An account of her history will be given in the following

Submitted for publication February 16 1965

The composition of the case material is shown in table I Three patients were men (aged 44—54 years) and 12 were women (aged 39—73 years) In every case various symptoms of malabsorption were present, in the form of anaemia, periodic diarrhoea, weight loss in several cases, and in about half of them severe dumping symptoms, of grade Pc 2—3 according to Meurling (20) Partial gastrectomy by the Billroth I method had been performed in two cases, whereas Billroth II resection had been done in the others In no case were there reasons to suspect chronic abuse of alcohol None of the patients had renal insufficiency, nor signs of any other metabolic or endocrine disease All the patients were ambulant, and all examinations were carried out in a special ward for metabolic studies

Controls These consisted of 15 patients (4 men and 11 women) who had recovered from some brief illness, or who were hospitalized for observation of some disease that was not serious The composition of the control series is seen in table II None of the patients had systemic disturbances all were ambulant and ready for discharge from hospital None had any signs of an endocrine or metabolic disorder Nor did any of them have any gastrointestinal disorder and none was being given medication of any importance in the present connection

Dietary history This was analyzed in collaboration with the department s dietician The patients were carefully questioned about their dietary habits, and about any intolerance to milk or cheese

Collection of food To obtain more exact data on the calcium content of the food customarily eaten by the patients and controls we used the following procedure During a period ranging from 2 to 4 days exactly the same quantity of food and drink as that taken in the individual case was collected daily in special dishes The calcium content of this food and drink was then determined chemically and in some cases the nitrogen content was also determined Both patients and controls were instructed to take as much as they wanted of the food and drink offered to them During this period, no medication was given, nor were any other investigations made

Faeces collections were marked by carmine, and each collection period comprised 6 days.

The *calcium* content of the diet was determined by the macro method of Shohl and Pedley (24)

The *nitrogen* content was determined by a modification of the Kjeldahl method

The *fat in the faeces* was determined according to van de Kamer et al (17) Normal value < 6 0 g/24 hours

The *nitrogen in the faeces* was determined by the same method as that used for the food Normal value < 2 0 g/24 hours

Alkaline phosphatase in serum was determined according to Buch and Buch (5) Normal range 2—7 units

Calcium infusion test This was performed according to Nordin and Fraser (22) For 4 days the patient is given a low-calcium diet (calcium content 140 mg/24 hours) The urinary excretion of calcium is determined and during the 3rd and 4th days the urine is collected in 12 hour portions On the 4th day, a solution of calcium gluconate in 500 ml of physiological saline is infused intravenously in the course of 4 hours the total amount of calcium administered being 15 mg/kg of body weight The calcium content of the urine during the first 12 hours is determined, and the basal value from the previous day is subtracted from it The difference is given as a percentage of the total quantity infused and is denoted as the net 12 hour urinary excretion of calcium The normal excretion is stated to range from 27 to 55 % (22) A value below 27 % implies an increased calcium requirement and is indicative of osteomalacia

Roentgenological examination of the skeleton was made in every case and covered the skull spinal column pelvis knee joints and hand joints

Results

The results are collected in tables I and II

TABLE I Laboratory data n 15 gastrectomized patients. B I Billroth I resect on B II Billroth II resect on

Case	Sex	Age (yrs)	Op	Se n alkal ne pl ospl a ase (Buch & Bucl un ts)	Ca fus on test Net 12 l r u nary e cre o ( )	Oral intake		Faeces	
						Ca	N	Fat	N
						(n g/day)	(g/day)	(g/24 l rs)	(g/24 l s)
1	ᵡ	51	B II	3 6	48 6	628	8 3	9 2	1 3
2		51	B II	6 5	17 8	252	—	20 6	2 5
3	ᵡ	59	B II	7 1	28 1	650	—	12 2	1 4
4		69	B II	4 7	20 3	410		3 6	1 2
5		56	B I	5 6	1 0	692		10 9	1 7
6		3	B II	11 7	24 8	800	7 8	11 5	1 8
7		56	B II	3 9	17 0			9 2	1 4
8		44	B II	5 3	38 0	795		3 8	
9		52	B II	5 6	33 6	465	8 8	6 6	0 7
10		53	B II	6 0	26 0	723		11 3	1 7
11	ɔ	54	B II	5 2	29 4	06	11 5	4 0	
12		52	B II	10 5	40 7	1 066		13 5	2 8
13		49	B II	5 9	48 6	193	9 8	1 7	
14	ɔ	54	B II	4 2	23 6	942		8 0	1 5
15		39	B I	1 8	46 0	753	8 3	7 7	1 3
Normal alue				2 7	27 55			6	<2 0

Calc um infus on test

Of the 15 gastrectom zed pat ents 7 1 man and 6 women) l ad a net 12 hour r nary calcium excretion of less than 27 o i e below tl e normal lower limit In o e case 5) tl e excret on was ex t emely low 1 % In tl e otl er 6 cases tl e val e ranged from 17 0 to 26 0 %

In 9 cases a normal value was re co ded i e tl e net 12 l our excret on ran ed from 28 1 to 48 6 %

Duri g tl e nfus on of calcium sl gl t l radycard a was consistently ol served l ut tl e ECG trac g sl owed no otl er otew rtl y feature A few patients had a sl ght headache and diures s was usu ally increased No other s de effects were noted

Alkal ne pho phata e

Two pat ents cases 6 and 12) had a ra sed value i e 11 7 and 10 5 u ts respectively In case 10 tl e borderl ne value of 6 0 un ts was recorded in the rema ning cases the value was normal

Faecal fat and n trogen content

In 8 cases tl e faeces had a h gh fat con te t tl e value rang ng from 9 2 to 20 6 g 24 hours In 3 (cases 9 14 and 15) the

The composition of the case material is shown in table I Three patients were men (aged 44—54 years) and 12 were women (aged 39—73 years) In every case various symptoms of malabsorption were present, in the form of anaemia, periodic diarrhoea, weight loss in several cases, and in about half of them severe dumping symptoms, of grade Pc 2—3 according to Meurling (20) Partial gastrectomy by the Billroth I method had been performed in two cases, whereas Billroth II resection had been done in the others In no case were there reasons to suspect chronic abuse of alcohol None of the patients had renal insufficiency, nor signs of any other metabolic or endocrine disease All the patients were ambulant, and all examinations were carried out in a special ward for metabolic studies

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Collection of food To obtain more exact data on the calcium content of the food customarily eaten by the patients and controls, we used the following procedure During a period ranging from 2 to 4 days exactly the same quantity of food and drink as that taken in the individual case was collected daily in special dishes The calcium content of this food and drink was then determined chemically, and in some cases the nitrogen content was also determined Both patients and controls were instructed to take as much as they wanted of the food and drink offered to them During this period, no medication was given, nor were any other investigations made

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Roentgenological examination of the skeleton was made in every case and covered the skull spinal column pelvis knee joints and hand joints

Results

The results are collected in tables I and II

view of the long observation period after operation a detailed account follows

Case 2 A 51 year-old woman born in 1912 A Billroth II partial gastrectomy had been performed in 1947 because of a peptic ulcer After operation she had slight dumping symptoms diarrhoea and a poor appetite and stated that she was never really hungry She could not tolerate milk or milk dishes and seldom ate cheese She ate only three cooked meals per week She drank tea in the morning and soda water at lunch and dinner Her diet evidently had an extremely low calcium content During the past 3 years she had lost 10 kg and on examination weighed 47 kg

Since July 1962 she had progressive muscular weakness in the proximal parts of her legs manifested as difficulty in getting up from a chair or out of bed Her gait had deteriorated and from the autumn of 1963 she found it difficult to manage stairs and therefore almost never left home Since Oct 1963 there was incipient weakness in the shoulders so that she found it hard to lift heavy objects She had suffered from numbness and a pricking sensation in her hands and feet since the autumn of 1962

On examination on June 1st 1964 she was exceedingly thin Marked paresis was present in proximal muscle groups especially in the legs There was bilateral atrophy of the shoulders upper arms, and gluteus and quadriceps muscles The Achilles tendon reflexes were decreased but otherwise the muscle reflexes were normal The plantar responses were flexor Superficial sensation was decreased in both feet

Laboratory data Serum calcium 7 1 mg 100 ml serum phosphorus 2 5 mg 100 ml alkaline phosphatase 6 5 units Urinary excretion of calcium 103 mg 24 hours Faecal fat content 0 6 g 24 hours Calcium infusion test and dietary analysis see table 1

Electromyography of right and left quadriceps muscle Dr I Kirstein myopathy

Biopsy specimen of right quadriceps muscle Dr K I Åstrum slight changes, possibly within normal limits

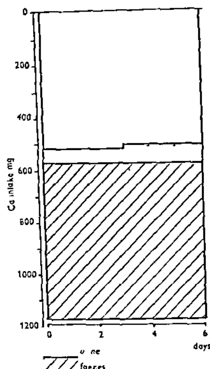


Fig 1 Metabolic study in 61 year-old man (ref 9) 6-day period Oral intake of calcium 1 193 mg/day The calcium balance was strongly positive and the patient retained about 500 mg of calcium a day

Roentgenological examination of the skeleton showed decalcification of the lumbar spine as well as around the finger joints and metacarpophalangeal joints

The diagnosis of osteomalacia was regarded as established The patient was given 140 000 I U of vitamin D (Fortedol) daily which produced a considerable improvement even after a few weeks She was able to move about more easily managed stairs better and started to go out and do her shopping Two months after institution of therapy the serum calcium was 9 5 mg/100 ml serum phosphorus 4 7 mg/100 ml and alkaline phosphatase 8 3 units

TABLE II Oral intake of calcium and nitrogen determinations in 15 non gastrectomized patients (controls)

Case	Sex	Age (yrs)	Oral intake Ca (mg/day)	Oral intake N (g/day)	Diagnosis
1	♂	35	1,135	11.2	Status post acute bronchitis
2	♂	44	828	12.8	Cephalalgia
3	♂	22	887	12.2	Spontaneous pneumothorax
4	♂	35	1 001	10.2	Myalgia
5	♀	58	1 094	11.2	Benign essential hypertension
6	♀	42	958	8.5	Status post acute pleurisy
7	♀	62	604	7.4	Dorsal insufficiency
8	♀	49	844	6.4	Status post bronchopneumonia
9	♀	62	772	6.6	Benign essential hypertension
10	♀	47	763	9.9	Angina pectoris
11	♀	69	1 030	9.1	Status post bronchopneumonia
12	♀	55	684	6.8	Chronic cystitis
13	♀	59	957	9.5	Status post thrombosis left lower leg
14	♀	62	736	7.2	Angina pectoris
15	♀	72	955	8.7	Aortic stenosis

value was slightly above the normal limit, whereas in the remaining 4 (cases 4, 8, 11 and 13) the fat content was less than 6 g/24 hours

The nitrogen content of the faeces was determined in every case in which the fat content was increased Two patients (cases 2 and 12) had a sligthly raised value, in the others the nitrogen content of the faeces was normal

Roentgenological examination of the skeleton

In 3 patients (cases 2, 4, and 5) generalized decalcification of the bones was observed, it was particularly marked in the vertebral column and the metacarpo phalangeal joints Only in case 2 could it be denoted as advanced No spontaneous fracture of Milkman's type was demonstrable in any of the cases

Dietary history

All the patients were unable to tolerate milk, and several of them cheese as well Symptoms of discomfort were also produced by food containing milk or flour, such as porridge, gruel, pancakes and puddings Most of the patients had a poorer appetite after operation than before it and some stated that they felt better when they did not eat About half of the patients had pronounced dumping symptoms even after small meals In some of them, the symptoms were so severe that they were brought on even by eating a few pieces of bread and butter All the patients had a feeling of discomfort in the epigastrium and nausea in connection with large meals

The symptoms were severe in case 2, since this case is of particular interest in

the skeleton is reduced by more than 50 per cent Consequently it is possible that a slight degree of osteomalacia and osteoporosis may have existed in some of our cases

As far as the pathogenesis of the disturbance in calcium metabolism after partial gastrectomy is concerned it has hitherto been ascribed chiefly to malabsorption from the intestine Malabsorption is common in patients who have undergone gastric resection and is stated to occur more often in those operated on by the Billroth II method than by the Billroth I method As early as *1946* Wollaeger et al (26) reported that an increased excretion of fat in the faeces was usual after Billroth II resection and that the excretion tended to be greater in those patients who had postcibal complaints Their results have been verified in subsequent investigations (11 12)

In our series steatorrhoea was present in 11 cases In 8 cases the faecal fat content ranged from 9 2 to 20 6 g/24 hours In most cases a normal amount of nitrogen was excreted in the faeces but in t a slightly raised value was recorded

The prerequisite for the occurrence of osteomalacia caused by steatorrhoea thus exists in a number of gastrectomized patients In many of them this has evidently been the pathogenetic factor as can be inferred from the good results of vitamin D therapy in several cases previously described 3 10 23

A low dietary intake of calcium as a cause of the disturbance in calcium metabolism after gastric resection has to the contrary been discussed to any extent in the literature

In an earlier paper we gave an account of a *61* year-old man who *6* years after partial gastrectomy *by the* Billroth II method was hospitalized because of weight loss and muscular weakness (9) He was unable to tolerate milk or cheese In a short term balance test he was found to have a strongly positive calcium balance with a spontaneous retention of 500 mg associated with a high calcium intake The high calcium retention could probably be explained by a previously low calcium intake (fig 1)

The Food and Nutrition Board recommends 800 mg as a suitable daily intake of calcium (13) It has on the other hand been stated that 500 mg a day should suffice to maintain the calcium balance in most adults 1

Malm 18) found in long term balance tests with a calcium intake of 450 mg 24 hours that 3 of 26 men were unable to adapt to such a low intake and that their calcium balance remained negative Some of the remainder did not become adapted until after 252 days

In the Scandinavian countries the average intake of calcium is over 1 000 mg per day the figure given for Sweden is 1 010 mg 1

Isaksson 16 found in an investigation of hospitalized patients that women consumed only 50—60 per cent of the calculated amount of various substances in the hospital diet The intake of men eating the ordinary hospital diet was on the contrary more than had been calculated Nine women given this diet consumed an average 800 mg of calcium a day which represents the quantity

Dietary analyses

The results of the dietary analyses in the gastrectomized patients are given in table I and those in the controls in table II

Gastrectomized patients In most cases, the calcium intake was lower than in the controls, and ranged from 199—1,066 mg/day (mean 649 ± 65 mg/day) Two patients (cases 2 and 13) had an extremely low intake, 252 and 199 mg/day, respectively In another two (cases 4 and 9) the intake was low, i e 410 and 465 mg/day No analysis of the dietary calcium and nitrogen was made in case 7 This patient stated that she ate almost nothing at home, and was admitted to hospital in an emaciated condition (weight between 35 and 37 kg) on several occasions In hospital she had a good appetite, and gained 5—7 kg in a few weeks Since dietary analyses would not have been representative in her case, they were omitted

Controls The calcium intake ranged from 604 to 1,135 mg day (mean 883 ± 41 mg/day) The difference between the mean calcium intake in the gastrectomized patients and in the controls is significant ($p < 0.01$)

Discussion

In recent years, disturbances in calcium metabolism have been found to be common after partial gastrectomy Thus Harvald et al (15) performed calcium infusion according to Nordin and Fraser (22) and noted pathological values in almost half of the patients in a selected series Manifest osteomalacia was present in three cases Deller and Begley (7) studied 100 gastrectomized patients and 100 controls Decalcification of the bones was twice as frequent in the former as in the latter Weight loss, anaemia, dumping symptoms and diarrhoea were more common in gastrectomized patients with skeletal changes than in those without them In 18 cases, there were reasons to suspect osteomalacia

Clark et al (6) found a disorder of calcium metabolism in 28 per cent of 55 operated patients In most cases, the disturbances were interpreted as osteomalacia, and in some it was considered to be combined with osteoporosis As in earlier investigations, we found a disturbance of calcium metabolism to be common in a selected series of partially gastrectomized patients A pathological value in a calcium infusion test was recorded in almost 50 per cent of our cases In one of them (case 2) additional laboratory data were indicative of osteomalacia In this case, the symptoms were severe, with progressive muscular weakness, as well as weight loss and diarrhoea Treatment with large doses of vitamin D resulted in regression of the muscular weakness, and the patient fairly soon regained improved mobility Her muscular symptoms are in good agreement with those in three cases described in a previous paper (10) We have concluded that the muscular weakness developed as a result of malabsorption after partial gastrectomy We have been unable to trace any earlier account of this form of myopathy in the literature

In most of our patients the density of calcium in the bones appeared normal at roentgenological examination No changes are however demonstrable roentgenologically until the calcium content of

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recommended by the Food and Nutrition Board (13).

In our controls as well, the actual intake of calcium (883 mg) was lower than that calculated at our hospital, i.e. 1,200 mg of calcium a day, and our results are thus in good agreement with Isaksson's findings (16).

In the present series, the mean daily calcium intake in the gastrectomized patients (649 mg) was significantly lower than in the control group, and also lower than the amount recommended by the Food and Nutrition Board.

Four of our patients had a calcium intake of less than 500 mg/24 hours. Case 4 is of particular interest; she had a normal amount of fat in the faeces but, despite this, a diminished urinary excretion of calcium in the infusion test. Her daily calcium intake was only 410 mg. It is possible that her low calcium intake was the root of the high calcium retention. In another patient (case 13), however, with an extremely low dietary calcium intake, the calcium infusion test was normal.

Analyses of the nitrogen content of the diet showed, in both the gastrectomized patients and the controls, values sufficient to cover the daily requirement of protein.

Thus, in the present investigation disturbances in calcium metabolism after partial gastrectomy were found to be common in a selected series, which is in agreement with earlier investigations. In several of our cases, the oral intake of calcium was found to be extremely low.

The results support the possibility of three causal mechanisms in a disturbance in calcium metabolism after partial gastrectomy. They are: 1. low oral intake of calcium, 2. steatorrhoea with malabsorption, and 3. a combination of these two factors.

Summary

An investigation was made in 15 patients who had undergone partial gastrectomy by the Billroth I method (2 cases) or the Billroth II method (13 cases). It consisted of a calcium infusion test according to Nordin and Fraser (22), analyses of the faecal fat and nitrogen content, dietary history, and determination of the dietary intake of calcium. The values for the calcium content of the diet were related to those in a control group of patients who had not undergone gastrectomy.

In 7 of the gastrectomized patients the calcium metabolism was pathological, with a decreased net 12-hour urinary excretion of calcium in the infusion test. In most cases the oral intake of calcium was low, and the mean value was significantly lower than in the control group.

The results indicate that a low dietary intake of calcium combined, in certain cases, with an increased faecal excretion of fat, can produce a calcium deficiency after partial gastrectomy.

Acknowledgement

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From the Card ovascular Depa tment (Head S en Akesson M D) Sodersjukhuset and the Ger atr c Un t S ureby Vårdhem (Head Fredr k Lund M D) Stockholm Sweden

Obliterative Arterial Disease of the Lower Limbs

IV Evaluation of Long term Anticoagulant Therapy

By

Carl Tillgren

Tl e effect of long term ant coagulant treatment on pat ents w th obl terative arterial diseases in the lower l mbs was reported by Lund n 1956 and later (4 5) H s pat ents are ncluded in the present extended study

In a prev ous paper (10) tl e course of tl e disease as descr bed as regards amputa ons myocard al nfarct ons n tracran al ascular les ons and surv val of d ffe ent groups of pat ents Tl e r g nal arter ographic f rd ngs total occlus o or stenoses only d d not seem to ha e m cl nfluence on surv val Nor d d pa e s lo ere subjected to amp at ap ear to ep esent a type of d ea e th a lo er surv al rate Ca n n ta t co nary l eart d ease (CHD le tl er han l caused a s gn f ca l er ng of tl e surv al rate II

I a pre ou paper 9 tl e cour e of the l l te e arte al d ea e n the fem al e as t d ed l y repeated te og apl es l at ents l o ere treat ed w tl a a la ts developed fe er total occlus ons and existing total occlu s ons seemed to grow less often than in pat ents v l o d d not receive anticoagu lants In the present paper a more de tailed analysis is made of these results Tl e influence of anticoagulant treatment on frequency of myocard al infarctions intracran al vascular les ons causes of death and survival rate w ll also be dealt v th

Material and methods

Data on 366 pat ents we e collec d from four hosp tals n S ockholm These pa ents had had s mp oms of ar er al nsuff c ency of the lo er l mbs befo e 1958 and were followed up un l e her the end of 1960 or the r death if this occurred p ev ously The observa on period began when he d agnos s of obl ter a e a erial disease n l e lower limbs was er f ed During l e observa on period 255 pa ents rece ed an coagulants whereas 111 d d not

S n e l 5 most pa ents who consul ed tl e Card ovascular Depa ment at Soder sjukf uset because of per pl eral ar erial diseases ha e been subjec ed o long erm anti oagulan l erapy Patients ncapable of

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TABLE I Observation months distributed according to control and anticoagulant (AC) time

	Control time before AC treatment	AC treatment			Control time after AC treatment	Total
		Effective	Ineffective	Unknown intensity		
Non-diabetics						
Male	8 341	3 916	1 458	823	2,205	16 743
Female	1 170	996	350	94	262	2 872
Diabetics						
Male	973	417	218	3	264	1 875
Female	401	35	32	18	0	486
Total	10 885	5 364	2 058	938	2 731	21 976

Total control time = 13 616 months
Total anticoagulant time = 8 360 months

observed values for respectively MPI and P P were plotted against time and joined by straight lines thus indicating the intensity of the treatment at any given period of time

Effective treatment was considered to have taken place when either the curve connecting the MPI values was below 61 or that connecting the P P values was below 26 %. When the curve exceeded these values the treatment was looked upon as being *ineffective*

For some patients it was not possible to obtain information on the prothrombin values for them the intensity of the anticoagulant treatment was stated to be *unknown*

Percentage of effective treatment was defined for each patient as the total periods of effective treatment in per cent of the total period of treatment

Classification of observation time

The patients were observed for 21 976 months (1 831 years) in all No anticoagulants were administered to 111 patients during the observation period 521 patients received anticoagulants for at most 3 months and 202 patients for more than 3 months.

The total period during which the patients were given anticoagulant treatment was 8 360 months (697 years) representing 38 % of the entire observation time (table I) The anticoagulant treatment was effective during 64 % of this time ineffective during 25 % and of unknown intensity during 11 %

Recording of complications in relation to anticoagulant treatment

In the arteriographic study development of total occlusions and appositional growth of existing total occlusions were recorded as complications "propagation" In patients treated with anticoagulants it was often not possible to state whether the propagation had occurred during effective or ineffective treatment Therefore the percentage of effective treatment was calculated for each individual observation period i e between two arteriographies The occurrence of propagation could thus be related to the efficiency of anticoagulant treatment

Most patients who suffered heart infarctions or cerebrovascular accidents were sent to a hospital As the prothrombin level was usually controlled on admission it was possible to determine whether the complication occurred during effective or ineffective anticoagulant treatment or during control time These complications will be reported only for the male non-diabetics in relation to the entire control time and to the time of anticoagulant treatment respectively

being adequately controlled, because of physical or mental invalidity, were excluded Patients with malignant hypertension, cancer or recent gastric ulcer with haemorrhage, were also excluded Not less than half of the 212 patients who were specially questioned about their history stated that they had suffered from stomach ulcer'

At the other departments in which patients were collected anticoagulants were used less often, therefore, most of these patients were used as therapy controls

Anticoagulants

The purpose of the anticoagulant treatment was to establish a hypoprothrombinaemia and to keep it as stable as possible on a therapeutic level during several years Consequently, long acting anticoagulants were used, and our experience in connection with these preparations was satisfactory (5) The following remedies were given

Dicumarol bishydroxycoumarin U S P AP® (Ferrosan, 50 mg) It requires 1—3 days for its action to develop and this then persists for a period up to 10 days after therapy is discontinued Following an initial dose of 200—250 mg, 50—100 mg were administered daily until the prothrombin time had reached an adequate therapeutic level The weekly maintenance dose then varied between 150 and 1 600 mg

Phenylpropyloxycoumarin U S P Marcoumar® (Roche a 3 mg) This preparation required 1—2 days for its action to develop which then persisted up to 18 days after withdrawal Following an initial dose of 15—18 mg, 6—12 mg were administered daily until the prothrombin time had reached an adequate therapeutic level The weekly maintenance dose varied between 9 and 60 mg

For persons over 60 years of age, AP was preferred to Marcoumar because of the former drug s more transient effect

Antidote

Phytonadione, U S P, Konakion® (Roche, 1 mg in 0 5 ml for injection, 1 mg in one emulsion drop for oral use, capsules, 10 mg) In cases of low prothrombin values or haemorrhagic complications 1—20 mg were given

Control of anticoagulant treatment

When beginning anticoagulant treatment, the patients, who were controlled ambulatory at Södersjukhuset and Sureby Vårdhem, received a printed memorandum supplying detailed information on the aim of the treatment its realization and the hazards involved The patients were especially instructed to contact the physician or the hospital immediately in case of haemorrhages, so as to control the prothrombin time to administer an antidote and to adjust the anticoagulant dose

The prothrombin level was controlled in most patients by the one stage method of Quick Lehmann (3) using a commercial thromboplastin (Ido kinas® Ferrosan) The normal prothrombin time was 20—21 seconds A microprothrombin index (MPI) was calculated as

$$\frac{\text{coagulation time in seconds for normal plasma} \times 100}{\text{coagulation time in seconds for patient plasma}}$$

The prothrombin proconvertin method (P P) of Owren and Aas (6) was used as an extra control in case of unexpectedly high or low values of MPI The P P values are expressed as per cent of the normal coagulation activity At Stureby Vårdhem only the P-P method was used

When beginning anticoagulant treatment the prothrombin time was controlled 2—3 times per week Later on prothrombin controls at intervals of 3—4 weeks were sufficient as long as the values were within the therapeutic region Intercurrent diseases dietary changes and medicines sometimes caused marked fluctuations of the values, the patients then had to be controlled more often

Intensity of anticoagulant treatment

The optimal therapeutic region was regarded as 40—60 when MPI was used and 10—25 % when P P was employed The

TABLE II Arter ograph c follow up Patients const tuting the r own controls

Pat ent s no	Age at beginning of observation	Observat on months	Propagat on (+/—)	Total occlus on: Orignal length in cm	Total occlus on: Appos tional growth n cm
A No total occlus on at beg nning of observation					
T ea ment per ods					
140 L	49	33			
1?? L	57	19	—		
73 R	8	26	—		
73 L	58	26	—		
Con rol per ods					
112 R	38	2			
140 L	46	30	—		
9 R	51	5	+		
73 L	52	80	—		
8 L	54	55	+		
? L	59	41	+		
73 R	60	18			
3 L	60	18	+		
B T tal occlus on at beg nn ng of observat on					
T ea ent per ods					
11 R	41	0		5	
7 R	51	17		21	—
9 R	2	53		1	
5 R	53	?8	+	21	2
R	6	25		1	22
R	57	19		2	
85 R	59	29		22	16
8 1	59	29		42	
Con rol per ods					
5 R	46	61		18	3
8 R	4	55		22	
R	59	41		2	21

1 Lef l b R R gh l nb Ital cs wo nan
Th s bserva on pe d was excluded as the leng h of the occlus on exceeded 27 cm

age was 53 years at the beginning of the bservati n peri d The total observa t n t me was 157 months The total occlus n ncreased in length during 2 observation periods and thus the propagation tendency was 12 7

The differences in propagation tend ency between the anticoagulant groups

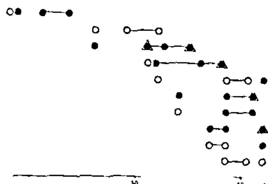


Fig 1 Patients constituting their own controls - - = control time without anticoagulant treatment, —— = anticoagulant treatment, ○ = arteriography revealed only stenosis ● = total occlusion, △ = appositional growth of total occlusion

Results

Arteriographic follow-up

In a previous paper (9) it was shown that total occlusions developed less often in patients who received anticoagulants than in those who were not given such treatment Existing total occlusions increased in length less often during anticoagulant treatment than during control time, but this difference was not statistically significant

A more detailed analysis of this material will be reported here with regard to

a Patients constituting their own controls,

b Patients with pronounced stenosis of the arteries and visible collateral vessels (arteriography group II),

c Efficiency of anticoagulant therapy

Development of total occlusions in previously only stenosed arteries and increase in length of preexisting total occlusions are reported as arteriographic propagation Propagation tendency' is defined as the number of patients showing propagation per 1,000 observation months (frequency per 100 observation months was given in the previous paper (9))

Patients constituting their own controls

Seven patients, in whom 10 lower limbs in all were investigated, had one or two observation periods during both anticoagulant treatment and control time There were 12 treatment and 11 control periods (table II, fig 1) in all

At the beginning of 4 anticoagulant periods no total occlusion existed The patients' mean age was 56 years at the beginning of the observation period The total observation time was 104 months No total occlusion developed during this time and thus the propagation tendency was 0 — At the beginning of 8 control periods no total occlusion existed The patients' mean age was 53 years at the beginning of the observation period The total observation time was 249 months During this time 5 total occlusions developed, and thus the propagation tendency was 20 1

At the beginning of 8 anticoagulant periods total occlusion existed One of these cases (85 L in table II B) was excluded as the occlusion exceeded 27 centimetres at the beginning of the observation period and such long occlusions never showed appositional growth in the present material (9) The patients mean age was 53 years at the beginning of the observation period The total observation time was 201 months The total occlusion increased in length during 3 observation periods and thus the propagation tendency was 14 9 At the beginning of 3 control periods total occlusion existed The patients' mean

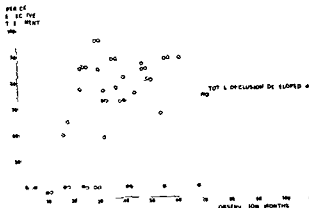


Fig 3 Development of total occlusions (●) related to the time of effective anticoagulant treatment in per cent of the whole of each observation period ○ indicates that stenoses only were present at the end of the observation period

for at least 80 per cent of the time in 17 observation periods The patients mean age was 57 (41—70) years at the beginning of the observation period These periods averaged 41 (24—62 total 702) months No total occlusions developed and thus the propagation tendency was 0

The treatment remained at an effective level for less than 80 per cent of the time in 15 observation periods The patients mean age was 57 (49—69) years at the beginning of the observation periods These averaged 32 (18—56 total 491) months Two total occlusions developed and thus the propagation tendency was 4 1

No treatment was given during 28 observation periods The patients mean age was 56 (30—66) years at the beginning of the observation periods These averaged 38 (2—131 total 1 077) months Thirteen total occlusions developed and thus the propagation tendency was 12 1

In the more homogeneous group comprising male non-diabetics aged 50—59 years the propagation tendency was 0 when the treatment was effective for at least 80 per cent of the observation periods 7 0 when effective for less than 80 per cent, and 9 3 when no treatment was given

The efficiency of the treatment during observation periods with pre existing total occlusions 1—27 cm in length is shown in fig 4 The treatment remained at an effective level for at least 80 per cent of the time in 16 observation periods The patients mean age was 55 (41—70) years at the beginning of the observation periods These averaged 33 (22—54 total 531) months During these periods 5 total occlusions increased in length and thus the propagation tendency was 9 4

The treatment remained at an effective level for less than 80 per cent of the time in 21 observation periods The patients mean age was 58 (42—67) years at the beginning of the observation periods These averaged 30 (2—63 total 620) months During these periods 7 total occlusions increased in length and thus the propagation tendency was 11 8

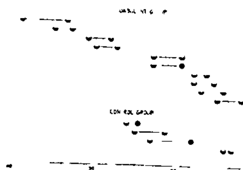


Fig 2 Patients with pronounced stenoses and visible collateral vessels (◒) at the beginning of the observation period, ● development of total occlusions

and the corresponding control groups are not statistically significant

Patients with pronounced arterial stenosis

Arteries with pronounced stenosis and visible collateral vessels (arteriography group II) are probably especially prone to develop total occlusions Therefore, such cases should be particularly suitable for studying the effect of anticoagulant treatment There were 13 patients in whom 14 lower limbs in all belonged to this group (table III, fig 2)

The anticoagulant group contained 9 patients in whom 10 legs were investigated The patients' mean age was 55 years at the beginning of the observation period The total observation time was 348 months Total occlusion developed in one limb during this time, and thus the propagation tendency was 2 9

The control group contained 4 patients in whom 4 legs were investigated The patients' mean age was 58 years at the beginning of the observation period The total observation time was 133 months Total occlusion developed in two limbs during this time and thus the propagation tendency was 15 0

TABLE III Arteriographic follow up Group II

Patient's no	Age at beginning of observation	Observation months	Propagation (+/—)
Treatment periods			
137 R	41	62	—
40 R	45	24	—
140 L	49	33	—
39 R	50	31	—
110 R	57	40	—
110 L	57	40	+
131 L	62	17	—
90 L	62	31	—
50 R	63	33	—
43 L	65	37	—
Control periods			
77 R	54	17	+
317 L	55	47	—
100 R	57	60	+
456 L	66	9	—

The groups were too small for statistical evaluation

Arteriographic propagation tendency related to efficiency of anticoagulant treatment

The prothrombin values were at a therapeutic level ' during varying parts of the anticoagulant periods The time of effective treatment was reported in per cent of the whole observation period between two arteriographies

The efficiency of the treatment during observation periods at the beginning of which stenoses only existed is shown in fig 3 The control periods are indicated in the lower part of the figure The treatment remained at an effective level

TABLE IV Myocardial infarctions during observation period Male non-diabetics without previous infarction

	Before anticoagulant treatment	During anticoagulant treatment			> 1 month after finishing anticoagulant treatment	Total
		Effective	Ineffective	Unknown intensity		
Patients with first infarction	15	1	7	*1	2	26
Observation months until infarction if any	7 296	2 807	1 003	690	1 779	13 575
Patients with infarction per 1 000 observation months	2 06	0 36	6 97	—	1 12	1 92

Frequency of infarction during anticoagulant therapy 2 00 during control periods 1 82
* Infarction without symptoms during anticoagulant treatment.

23 total 169) cm and the appositional growth was 3 1 (4—16, total 49) cm The mean observation period was 33 (22—54 total 531) months Thus, the appositional growth was 91 cm per 1 000 observation months

When the anticoagulant treatment was effective for less than 80 per cent of the observation period 7 out of 21 occlusions increased in length The original length averaged 13 0 (1—27 total 273) cm and the appositional growth was 5 7 (2—33 total 119) cm The observation period was 30 (2—63 total 620 months Thus the appositional growth was 19 1 cm per 1 000 observation months

When no anticoagulant treatment was given 12 out of 22 total occlusions increased in length The original length averaged 11 9 (1—27 total 261) cm and the appositional growth was 8 0 (3—35 total 177) cm The observation period was 60 (4—102 total 800) months Thus, the appositional growth was 201 cm per 1,000 observation months

The difference in appositional growth between these three groups was not statistically significant

Myocardial infarctions during observation period

Out of 223 male non diabetics with no previous myocardial infarction 26 developed this complication during the observation period 15 before anticoagulant treatment was administered 9 during treatment and 2 after treatment was concluded (table IV) Thus 17 infarctions occurred during the control time and 9 when anticoagulant treatment was given The number of patients with their first infarction per 1 000 observation months was 1 82 during the control periods and 2 00 during the periods of anticoagulant treatment

The frequency of infarction was lower during effective anticoagulant treatment

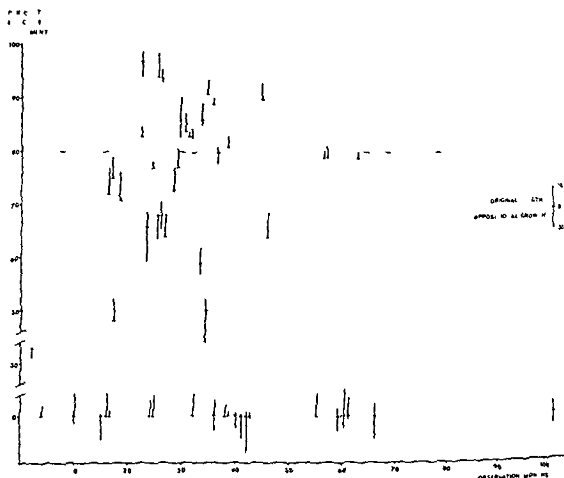


Fig 4 Appositional growth of total occlusions (‾) related to time of effective anticoagulant treatment in per cent of the whole of each observation period Original length of total occlusion indicated by ( )

No treatment was given during 22 observation periods The patients mean age was 55 (40—64) years at the beginning of the observation periods These averaged 40 (4—102 total 880) months During these periods 12 total occlusions increased in length and thus the propagation tendency was 13 6

In the group comprising male non diabetics aged 50—59 years the propagation tendency was 9 9 when treatment was effective for at least 80 per cent of the observation periods 11 8 when effective for less than 80 per cent and 13 2 when no anticoagulant therapy was given

The differences in propagation tendency were not significant between any of these groups

Appositional growth in cm of existing total occlusions

When the anticoagulant treatment was effective for at least 30 per cent of the observation period 5 out of 16 total occlusions increased in length (fig 4) The original length averaged 10 5 (1—

TABLE IV Myocardial infarctions during observation period Male non-diabetics without previous infarction

	Before anticoagulant treatment	During anticoagulant treatment			>1 month after finishing anticoagulant treatment	Total
		Effective	Ineffective	Unknown intensity		
Patients with first infarction	15	1	7	*1	2	26
Observation months until infarction if any	7 296	2 807	1 003	690	1 779	13 575
Patients with infarction per 1 000 observation months	2 06	0 36	6 97	—	1 12	1 92

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* Infarction without symptoms during anticoagulant treatment.

23 total 169) cm and the appositional growth was 3 1 (4—16 total 49) cm The mean observation period was 33 (22—54 total 531) months Thus the appositional growth was 91 cm per 1,000 observation months

When the anticoagulant treatment was effective for less than 80 *per cent* of the observation period, 7 out of 21 occlusions increased in length The original length averaged 13 0 (1—27 total 273) cm and the appositional growth was 5 7 (2—33 total 119) cm The observation period was 30 (2—63 total 620) months Thus the appositional growth was 191 cm per 1 000 observation months

When no anticoagulant treatment was given 12 out of 22 total occlusions increased in length The original length averaged 11 9 (1—27 total 261) cm and the appositional growth was 8 0 (3—35 total 177) cm The observation period was 40 (4—102 total 200) months Thus the appositional growth was 201 cm per 1,000 observation months

The difference in appositional growth between these three groups was not statistically significant

Myocardial infarctions during observation period

Out of 223 male non-diabetics with no previous myocardial infarction, 26 developed this complication during the observation period 15 before anticoagulant treatment was administered 9 during treatment and 2 after treatment was concluded (table IV) Thus 17 infarctions occurred during the control time and 9 when anticoagulant treatment was given The number of patients with their first infarction per 1 000 observation months was 1 82 during the control periods and 2 00 during the periods of anticoagulant treatment

The frequency of infarction was lower during effective anticoagulant treatment

TABLE V Intracranial vascular lesions (IVL) during observation period Male non diabetics without previous IVL

	Before anticoagulant treatment	During anticoagulant treatment			>1 month after finishing anticoagulant treatment	Total
		Effective	Ineffective	Unknown intensity		
Patients with first IVL	11	1	4	—	6	22
Observation months until IVL if any	8,098	3 483	1,336	816	2,191	15,924
Patients with IVL per 1,000 observation months	1 37	0 29	3 0	—	2 74	1 38

Frequency of IVL during anticoagulant therapy 0 89, during control periods 1 65

than during the control time, but it seemed to be considerably higher during ineffective treatment than during the control time The differences are not statistically significant due to small numbers

Intracranial vascular lesions during observation period

Out of 254 male non diabetics with no previous intracranial vascular lesion (IVL), 22 developed this complication during the observation period, 11 before anticoagulant treatment was administered, 5 during treatment, and 6 after treatment was concluded (table V) Thus, 17 IVLs occurred during the control time and 5 when anticoagulant treatment was given The number of patients with their first IVL per 1,000 observation months was 1 65 during the control periods and 0 89 during anticoagulant therapy

One out of 17 IVLs occurring during the control time was known to be an intracerebral haemorrhage, whereas 2 out of 5 IVLs during anticoagulant treatment were subdural haematomas One of these occurred during effective and the other during ineffective anti coagulant therapy

The frequency of IVL was lower during effective anticoagulant treatment than during the control time, but it was higher during ineffective treatment than during the control time The differences are not statistically significant

Survival rate for patients treated with anticoagulants compared with that for the controls

This comparison was confined to male non diabetics aged 60—69 years at the beginning of the observation period

The anticoagulant group consisted of 28 patients who were treated with anticoagulants from the beginning of the observation period Patients were also included whose treatment was started during the first 3 observation months

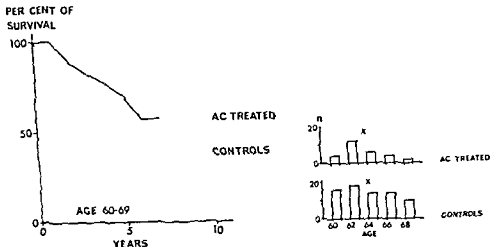


Fig. 5. Percentage of survival for male non-diabetics, 60—69 years ——— = anticoagulant group control group. Age distribution to the right. n = number of patients $\bar{x}$ = mean age.

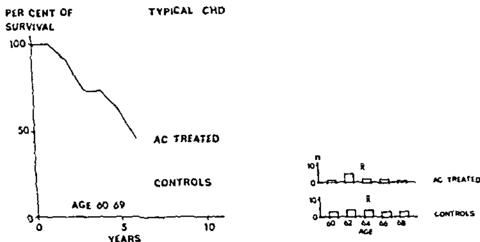


Fig. 6. Percentage of survival for male non-diabetics 60—69 years, with concomitant typical coronary heart disease at the beginning of the observation period ——— = anticoagulant group = control group. Age distribution to the right. n = number of patients, $\bar{x}$ = mean age

Two patients who died 1 and 3 months respectively after termination of anticoagulant treatment were regarded as having died during anticoagulant treatment.

The control group comprised 72 patients who had received anticoagulant treatment either for less than 3 months or not at all and had not died during this treatment. The 20 patients who

TABLE VI Male non-diabetics 60—69 years

	Anticoagulant group	Control group
Number of patients	28	72
Age at beginning of observation period	63 8	64 2
Observation months	46 5 (14—74)	38 7 (2—143)
Cardiac findings at beginning of observation period		
Normal	10	29
Possible CHD	5	12
Typical CHD	11	17
Unknown	2	14

began anticoagulant treatment only more than 3 months after the beginning of the observation period were regarded as controls until the beginning of the anticoagulant treatment

The survival curves were calculated according to the life table method (2) The one for the control group was steeper than that for the anticoagulant group (fig 5), but the difference between the curves was not significant for any point on the curves

Table VI shows the composition of the two groups, especially as regards the cardiac condition at the beginning of the observation period Normal cardiac findings were about equally common in both groups, but typical coronary heart disease (CHD) was more common in the anticoagulant group Consequently, on the basis of the results reported on in a previous paper (11) the prospect of survival was less favourable for the anticoagulant group than for the control group In view of this finding, survival curves were calculated for the two groups with typical CHD at the beginning of the observation period (fig 6) The anticoagulant group comprised 11 patients and the control group 17 patients These curves showed a more pronounced difference than those in fig 5, but the groups were too small to permit statistical analysis

Causes of death in male non diabetics

Acute cardiac death occurred in 23 out of 71 patients (32 %), 12 of these had signs of recent coronary thrombosis and or recent myocardial infarction and, therefore, the cause of death was regarded as acute myocardial infarction, the other 11 patients exhibited only arteriosclerotic changes in the coronary arteries and often signs of old but no recent infarctions and, therefore, the cause of death was classified as "acute arteriosclerotic heart disease" (table VII)

Out of the 12 myocardial infarctions, 9 occurred during the control time, 2 during ineffective, and only 1 during effective, anticoagulant treatment The latter patient had had two previous infarctions and died on a holiday tour Autopsy performed at a county hospital, disclosed pulmonary œdema and a large infarction in the posterior wall" Nothing was stated about the age of the infarction or coronary thrombosis Therefore the cause of death in this patient could just as well have been classified as acute arteriosclerotic heart disease

Out of the 11 patients who died of acute arteriosclerotic heart disease, 5 deaths occurred during the control time, 5 during effective treatment, and 1

TABLE VII Causes of death in male non-diabetics

	Before anticoagulant treatment	During anticoagulant treatment			>1 month after finishing anticoagulant treatment	Total
		Effective	Ineffective	Unknown intensity		
Arteriosclerotic heart disease						
Acute	2	5	—	1	3	11
Chronic	—	2		—	2	4
Myocardial infarction	6	1	2	—	3	12
Cerebral thrombosis	4		2	—	3	9
Other vascular diseases	5	—	—	1	4	10
Haemorrhages						
Intracranial	1	1	1		—	5
Other		1	1	—	—	
Infections, postoperative deaths etc	2	2	2		2	8
Cancer	5	1	—	—	6	12
Total	25	13	8	2	23	71
Observation months	8 341	3 916	1 458	823	2 205	16 743

TABLE VIII Causes of death per 1 000 observation months. Male non-diabetics

	Control time	Anticoagulant time			Total
		Effective	Ineffective	Unknown intensity	
Acute arteriosclerotic heart disease	0 47	1 28		1 22	0 97
Myocardial infarction	0 85	0 23	1 37		0 48
Total acute cardiac death	1 33				1 45
Haemorrhages	0 12	0 51	1 37		0 65

during anticoagulant therapy of unknown intensity.

Cerebral thrombosis was the cause of death in 9 patients, 7 occurred during the control time and 2 during ineffective anticoagulant treatment.

Intracranial haemorrhage occurred in 3 patients, in one during the control time, in another during effective treatment (cerebral) and in the third during ineffective treatment (subdural).

Other haemorrhages occurred in 2 patients, in one during effective treatment (duodenal ulcer) and in the other during ineffective treatment (retroperitoneal

haemorrhage after thromboendarterectomy of the iliac artery).

When comparing the anticoagulant group and the control group, the number of patients whose death was ascribed to a certain cause was calculated per 1,000 observation months (table VIII). Acute cardiac death was almost equally as common in both groups, but myocardial infarction was more common during the control time, and "acute arteriosclerotic heart disease" more common during anticoagulant treatment.

With regard to haemorrhagic deaths, these were more common during anticoagulant treatment, as could be expected. They seemed to be more common during ineffective than during effective treatment. This is probably due entirely to chance, as the number of patients was small.

Discussion

Propagation tendency of obliterative changes, i.e. development of total occlusions and appositional growth of existing, total occlusions in the femoral region was studied by means of repeated arteriographies. This tendency was found to decrease during anticoagulant therapy as compared with that during periods when such treatment was not administered.

The blood flow through the lower limbs depends also on factors extraneous to the femoral region. Decreased cardiac output and blood pressure as well as obliterative changes in the aorta, the iliac, tibial and fibular arteries and the veins can diminish the blood flow and increase the risk of thrombotic development in the femoral region. Appositional growth of existing occlusions can readily occur as far as the points where large collaterals leave or enter the artery. It was impossible to evaluate the influence of the above mentioned factors on the propagation tendency, as the investigations were incomplete in many respects. Consequently, the present report can deal adequately only with the influence of anticoagulants on the local arterial changes.

The *development of total occlusions* was less pronounced during anticoagulant therapy than during the control period for the entire material of patients (** significance) as well as for groups with pronounced stenoses (arteriography group II), patients constituting their own controls and male non-diabetics aged 50—59 years. These subgroups were too small for statistical evaluation. No total occlusion developed during observation periods when the anticoagulant treatment had been effective during 80 per cent or more of the time.

Appositional growth of existing total occlusions was influenced less by anticoagulant therapy. There was no statistically significant difference between the anticoagulant and the control group in any of the groups of patients investigated, but in all except one of these groups the propagation tendency was lower during anticoagulant treatment.

Effective anticoagulant treatment during 80 per cent or more of the observation period seemed to decrease the number of existing occlusions that had grown and also the length of appositional growth, but the difference between the most adequately treated group and the controls was not statistically significant.

To sum up, the local effect of anticoagulant treatment, as studied in the femoral region was statistically significant as regards the development of total occlusions This was particularly evident when the prothrombin values were on an effective therapeutic level for at least 80 per cent of the observation periods Existing total occlusions increased less measured in centimetres when the treatment was adequate to this extent This may indicate a favourable delay of the propagation of the thrombotic process although the difference between the anticoagulant and the control groups was not statistically significant

When *myocardial infarctions* and *intracranial vascular lesions* occurred, the patients were sent as a rule to a hospital where the prothrombin level was usually *controlled* on admission Thus it could be ascertained whether in patients treated with anticoagulants the complication had taken place during effective or *ineffective treatment* The frequencies *of these* complications were calculated only in respect of the first infarction and intracranial vascular lesion for each patient and then related to total *control time* total anticoagulant time *and* periods of effective ineffective and *unknown* intensity of anticoagulant treatment

The frequency of *infarctions* was almost the same during both the total *control* time and the anticoagulant time A *low frequency* during the period of effective treatment indicated what could be achieved by an adequate treatment The very much higher frequency during ineffective treatment is perhaps not exclusively due to this A gradual deterioration of the physical and mental capacity of the patient may have made it impossible for him to take the medicine and to come regularly to the controls It is always difficult to judge which is the cause and which the effect of unsatisfactory results of treatment

The frequency of intracranial vascular *lesions* resembled that of myocardial infarctions Both these complications were too few to show statistically significant differences between various observation periods

The *survival curve* for patients treated with anticoagulants had a better course than that for control patients in the same age group The difference became more pronounced when only patients with concomitant coronary heart disease were compared The groups of patients however were too small to show statistically significant differences between the curves Nevertheless it is interesting that anticoagulant treatment seems to be especially useful for patients with peripheral arterial disease and concomitant coronary heart disease

As regards the *causes of death* a probable effect of anticoagulant treatment could be demonstrated Sudden death seemed to be equally common in both the anticoagulant and the control groups *but recent coronary thrombosis with* recent necrosis of the myocardium was a frequent cause in the control *group* and a rare cause in the anticoagulant group Haemorrhages *intracranial or intestinal* were *more common during* anticoagulant *treatment* but curiously *enough most of them* occurred during ineffective treatment

haemorrhage after thromboendarteriectomy of the iliac artery)

When comparing the anticoagulant group and the control group, the number of patients whose death was ascribed to a certain cause was calculated per 1,000 observation months (table VIII) Acute cardiac death was almost equally as common in both groups, but myocardial infarction was more common during the control time, and "acute arteriosclerotic heart disease" more common during anticoagulant treatment

With regard to haemorrhagic deaths, these were more common during anticoagulant treatment, as could be expected They seemed to be more common during ineffective than during effective treatment This is probably due entirely to chance, as the number of patients was small

Discussion

Propagation tendency of obliterative changes i e development of total occlusions and appositional growth of existing total occlusions in the femoral region was studied by means of repeated arteriographies This tendency was found to decrease during anticoagulant therapy as compared with that during periods when such treatment was not administered

The blood flow through the lower limbs depends also on factors extraneous to the femoral region Decreased cardiac output and blood pressure, as well as obliterative changes in the aorta the iliac, tibial and fibular arteries and the veins can diminish the blood flow and increase the risk of thrombotic development in the femoral region Appositional growth of existing occlusions can readily occur as far as the points where large collaterals leave or enter the artery It was impossible to evaluate the influence of the above mentioned factors on the propagation tendency, as the investigations were incomplete in many respects Consequently, the present report can deal adequately only with the influence of anticoagulants on the local arterial changes

The *development of total occlusions* was less pronounced during anticoagulant therapy than during the control period for the entire material of patients (** significance) as well as for groups with pronounced stenoses (arteriography group II), patients constituting their own controls and male non diabetics aged 50—59 years These subgroups were too small for statistical evaluation No total occlusion developed during observation periods when the anticoagulant treatment had been effective during 80 per cent or more of the time

Appositional growth of existing total occlusions was influenced less by anticoagulant therapy There was no statistically significant difference between the anticoagulant and the control group in any of the groups of patients investigated but in all except one of these groups the propagation tendency was lower during anticoagulant treatment

Effective anticoagulant treatment during 60 per cent or more of the observation period seemed to decrease the number of existing occlusions that had grown and also the length of appositional growth but the difference between the most adequately treated group and the controls was not statistically significant

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7 SELVAAG O Langtidsbehandling med antikoagulantia ved arterioscleroiske sykdommer i ekstremiteterne (Norwegian) Nord med 61 151 1959

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10 TILLGREN C Obliterative arterial disease of the lower limbs II A study of the course of the disease Acta med scand 178 103 1965

11 TILLGREN C Obliterative arterial disease of the lower limbs III Prognostic influence of concomitant coronary heart disease Acta med scand 178 121 1965

Summary

The effect of long-term anticoagulant treatment was studied in patients with obliterative arterial disease in the lower limbs This was done both locally, in the femoral region, by means of repeated arteriographies, and generally, in respect of frequency of heart infarctions and intracranial vascular lesions, survival rate, and causes of death

In all, 366 patients who, before 1958, displayed symptoms of arterial insufficiency in the lower limbs were followed-up to the end of 1960 or until their death if this occurred previously During the observation period anticoagulants were given to 202 patients for 3 or more months, whereas 164 patients either received no anticoagulants at all or only for a period less than 3 months

The number of total occlusions that developed in stenosed arteries was smaller (**significance) during anticoagulant periods than during control periods Existing total occlusions increased in length less often (not significant) during anticoagulant treatment than during control time The increase of total occlusions, as measured in centimetres, seemed to be smaller the more effective the anticoagulant treatment

Only one patient suffered a first myocardial infarction during effective anticoagulant treatment The frequency was larger when no anticoagulants were given but largest during ineffective treatment The frequency of infarction was almost the same for the total period of treatment and the total period of control As regards intracranial vascular lesions the tendency appeared to be the same

The survival curve for an anticoagulant group showed a better course than that for a control group The difference became more pronounced when two groups with concomitant coronary heart disease were compared The difference between the curves, however, was not statistically significant

Cardiac death was common in both anticoagulant and control group Recent coronary thrombosis and/or recent myocardial necrosis was common in the control group but rare in the anticoagulant group Haemorrhagic deaths were more common in the anticoagulant group The material was too small for statistical evaluation

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Nocardiosis During Steroid Treatment of Auto-immune Haemolytic Anaemia

Report of a Fatal Case[1]

By

K Gydell, I Juhlin, H Ljunggren, J G Nordén and B Fors

Systemic nocardiosis has been reported with increasing frequency in recent years (7 28 34) The condition is important particularly in the differential diagnosis of pulmonary affections (29 30, 38 39) as well as of diseases of the central nervous system where it is feared and not uncommonly fatal (23 25 40) So far only a few cases of nocardiosis have been reported in the Scandinavian literature 24

Case report

The patient was a 4[illegible] year-old married woman Until the onset of her disease in 1961 she worked at a local shoe factory On routine mass chest X ray of the personnel of the factory in 1955 [illegible] the woman was 34 years old a small infiltrate was detected in the upper part of the left lung At that time the patient felt well Tuberculosis had occurred earlier in the patient's family One sister had died from pulmonary tuberculosis when the patient was 16 years old In view of the X ray finding in 1955 the patient was followed up with roentgen examination once a year

At such an examination on July 27 1961 the chest X ray was unchanged but the E S R was found to be 144 mm/1 hour There was slight anaemia Hb 11 2 g/100 ml The patient reported that during the spring of 1961 she felt tired but did not think it was anything serious In the beginning of Aug 1961 the woman noticed that the whites of the eyes had become yellow and she sought medical advice The blood values were found to be decreased On Aug 23 the patient was admitted to the medical department Central Hospital Halmstad She was found to have pronounced anaemia Hb 5 5 g/100 ml R B C 1 4 mill mm³ W B C 4 000 mm³ reticulocytes 13 % The serum bilirubin was increased The serum contained no demonstrable haptoglobin Coombs direct test was positive Electrophoresis slightly increased gammaglobulin Slight roentgenographic enlargement of the liver and spleen

Submitted for publication February 18, 19[illegible]

[1] Read in abbreviated form at the Swedish Pathological Society December 1 1962 (15)

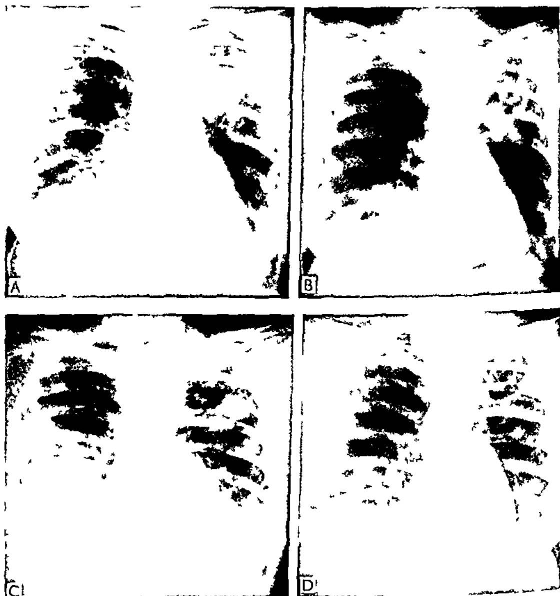


Fig 1 A 23 II 1962 Outside and above left hilum parenchymal changes with few areas of cavitation Widespread parenchymal changes and atelectases in middle lobe of right lung B 8 III 1962 Infiltration now wider and cavities larger C 1 IV 1962 Cavities in left lung now confluent D 30 IV 1962 Parenchymal changes in apex of left lung and base of right lung regressed but some cavities persist

The anaemia was thus obviously of haemolytic type, and steroid treatment was began with prednisolone (Deltacortril Pfizer) in a dose of 40 mg a day on Aug 29 1961 The blood values improved and the patient left hospital on a maintenance dose of 10 mg a day After only one month, however the patient again became worse and was re admitted to the hospital The prednisolone dose was increased and the blood values again responded favourably Guinea pigs inoculated with gastric aspirate gave a negative finding for tuberculosis The chest X ray was unchanged

On Jan 25 1962 the patient was for the third time admitted to the hospital because of chills fever and slight non productive cough Chest X ray now showed an infiltrate

on the upper part of the left lung It was diffusely outlined but could be distinguished from the previously known lesion in that lung A further change was also observed in the lower part of the right lung *During the* following week the changes in the left lung progressed rapidly After a further week during which the fever persisted respiratory tract symptoms appeared Signs of moniliasis were now seen in the oral cavity This suggested that the pulmonary affection might be due to a fungus infection The patient was treated with mycostatics and the changes in the oral cavity regressed The patient was still anaemic Because of the fever the steroid dose was increased From Aug 23 1961 to Feb 20 1962 the patient had received on the average 20 mg prednisolone a day

On Feb 20 1962 the patient was transferred to the Department of Internal Diseases Malmö General Hospital Due to earlier steroid treatment the patient had a Cushingoid habitus Her general condition was good but she was slightly short of breath and had a productive cough with a daily removal f 50—110 ml of mucopurulent material Physical examination of the heart revealed a pericardial rub in the left sternal margin in I_3—I The rhythm was regular and the rate 104 Electrocardiography showed no definite abnormalities Liver and spleen were not palpable A deep-seated lymph node was palpated in the left axilla

Chest X ray (fig 1 A immediately after admission to hospital now showed that the parenchymal changes in the left lung were n lergoi g ca a on Repeated examina t s of the sputum f r tubercle bacilli gave negative results The Mantoux reaction with a dose of 1 g was negative

Haematological data Hb 7 8 g 100 ml R B C ?2 million ^{3} W B C 10 400 mm^3 Diff N 66 E 0 5 B L) M 4 5 % Re loc tes 6 000 mm^3 Serum bilirubin 1 0 g 100 1 D R negative COHb in blood 1 6 ncreased value indicating hyperhaemolysis Urobilinogenuria No l lrulan a Coombs direct test positive S gl 1 reased osmotic fragility of the red l s The haematological data thus confirmed the diagnosis of *acquired haemolytic anaemia* Attention was now concentrated on the pulmonary lesions Since repeated direct examination of the sputum for tubercle bacilli had proved *negative* tuberculosis was considered less likely The roentgen appearance would not allow exclusion of a primary or secondary pulmonary tumour The patient was therefore subjected to bronchoscopy (March 6 1962) But no signs of bronchial tumour were detected On that occasion bronchial secretion was collected for culture which gave abundant growth of *Nocardia asteroides* Sputum samples collected shortly before this examination gave growth of Nocardia as well as of Monilia Repeated culture of the blood and faeces for Nocardia proved negative

Bacteriological examinations

The microorganisms were strictly aerobic and required no CO_2 stimulation Good growth occurred at 37 C as well as at 28 and 22 C but was most rapid at 37 C Not until after 72 hours incubation were colonies easily observed with the naked eye Counting of the colonies in a low power stereo microscope showed an increasing number of colonies during a period of 10 days

	2 days	5 days	10 days
Sample A	0	14	0 colonies
» B	0	0	> 200 »

Colony characteristics after 3—5 days incubation

On *blood agar* the colonies were 0 25—1 0 mm in diameter circular with undulate margin wrinkled with granulated shiny surfaces The colonies were hard and firmly attached to the medium They were greyish white at first but gradually *became orange yellow* After 5 days there was abundant but short aerial mycelium visible only under x 10—40 magnification

On *H IP medium* 20 the colonies were up to 2 n n diameter markedly folded and shiny Pigment formation was stronger than on any of the other media but aerial mycelium was lacking

On *H IP medium* 9 growth was slower than on blood agar but pigment formation

TABLE I The number of colonies growing after treatment of a suspension of Nocardia in saline with equal volumes of acid or alkali during different periods of time at room temperature The suspensions were neutralized filtered through a membrane filter (Membranfilter Tb 2, Gottingen) and incubated at 37° C for seven days

Dilution	Controls Untreated suspensions	Suspensions treated with equal volumes of							
		6 vol % sulphuric acid				4 vol % sodium hydroxid			
		1 min	3 min	5 min	10 min	1 min	3 min	5 min	10 min
$1/10^4$	Confluent growth	100–200	100–200	100–200	100–200	9	0	0	0
$1/10^5$	Almost confluent growth	75	50	40	20	0	0	0	0

TABLE II Determination of sensitivity to antibiotics with the disc diffusion method on blood agar

	Zone (mm)	MIC	
Sulpha	56	0 1 mg %	Sensitive
Penicillin	10	40 IU/ml	Slightly sensitive
Streptomycin	19	8 mcg/ml	Fairly sensitive
Tetracyclin	14	6 mcg/ml	Slightly sensitive
Chloramphenicol	16	25 mcg ml	Slightly sensitive
Erythromycin	15	20 mcg ml	Slightly sensitive
Kanamycin	0	100 mcg ml	Slightly sensitive

was strong, and short aerial mycelium was abundant

On *McLeod's medium enriched with 25 % ascitic fluid* no aerial mycelium could be seen

On *tryptose agar and cryptococcal capsule medium* (26) growth was slow but short aerial mycelium was abundant

On *Sabouraud agar* containing 20 IU penicillin and 50 mcg streptomycin per ml no colonies grew

On *Löwenstein Jensen medium* the colonies were 0 25—1 5 mm in diameter after 3 days incubation They were circular with an undulate margin wrinkled and shiny but coarsely granular After 14 days incubation the colonies were up to 5 mm in diameter and their surfaces were more wrinkled and irregular They were b[illegible] like M tuberculosis Despite prolonged incubation the formation of aerial mycelium was poor

In sputum as well as in other materials the dominating colony was the above-mentioned rough type There were however also a few strongly yellow smooth variants, which only after long incubation showed a poor aerial mycelium

Gram staining of the two varieties showed long branching gram positive filaments and rods of varying length Ziehl Nielsen staining of the colonies grown on blood agar WAP or HAP medium rarely showed any acid fast elements while direct smears from bronchial secretion and colonies grown on Löwenstein Jensen medium in 20—50 % contained acid fast rods and acid fast fragments of threads

TABLE III Comparison of physiological characteristics. Figures indicate per cent positive strains 13. S 127 ro and S 127 sm are the two variants isolated from the patients

Property		S 127 ro		S 127 sm		N 333		N asteroides	N 318		N caviae	N corallina		N corallina M rhodochrous	N brasiliensis
Decomposition of		22	37	22	37	22	37		22	37		22	37		
Casein	*	−		−	−	−		0		−	0	−		0	98
Gelatin			−	+	+	−			−	−				3	
Tyrosine	*				−			2			0	+	+	73	100
Xanthine					−	−		0		+	100			0	0
Hypoxanthine	*				−	−	−	4	+	+	100				94
Hydrolysis of starch					−		−	56			60			97	55
Acid from															
Glycerol	*	+	+	+	+	+	+	99	+	+	100	+	+		98
Erythritol	*						−	8			0			0	0
Adonitol	*			−		−	−	0	−	−	0				0
Sorbitol							−	0			0	+	+	100	0
Dulcitol									−					0	
Mannitol	*					−	−	1	+	+	100	+	+	100	94
Inositol	*					−		3	+	+	100			12	100
Arabinose								0			0			0	0
Rhamnose		+	+	+	+	+	+	32			0			3	0
Xylose	*				−			0			0			8	0
Glucose	*	+	+	+	+	+	+	97	+	+	100	+	+	97	97
Galactose					+			24			0			3	92
Mannose								16		+	47	+		100	68
αMethyl-D-Glucoside	*							0			0			0	0
Lactose	*			−		−	−	0			0			0	0
Maltose								7			87			16	4
Trehalose		+	+		+	+	+			+				93	
Raffinose	*			−				0			0			0	0
Utilization of															
Acetate	*	+	+	+	+	+	+	100	+	+	100	+	+		100
Propionate	*	+	+	+	+	+	+	100	+	+	100				100
Lactate		−			+	+	+	31				+		100	
Pyruvate			−		+	+	+	99			100		+		100
Oxalate		−			−		−							0	
Succinate	*	+	+	+	+	+	+	94	+	+	100	+	+	100	100
Malate	*	+	+	+	+	+	+	95	+	+	100			98	100
Tartrate		−	−	−	−		−							0	
Citrate			−	−	−	−		34			67	+	−	87	93
Benzoate		−	−	−		−	−	0			0		−	80	2
Nitrite from Nitrate		+	+	+	+	+	+	88	+	+	100	+	+	83	90
Urease		+	+	+	+	+	+		+	+		−	+		

* According to Gordon & Mihm these reactions are most important. Variation by a strain in any one or two of them does not preclude its identification as N. asteroides.

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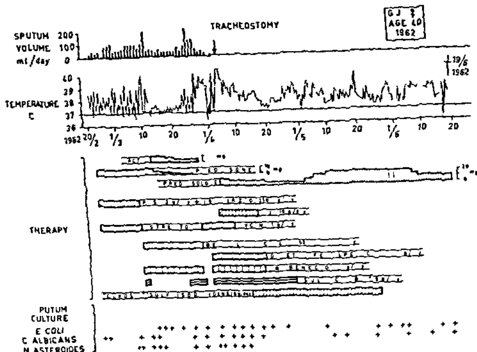


Fig 2 Course and treatment.

reticulocytes which was formerly markedly increased to 50 000 and 90 000/mm³ fell to 0 to 2 000/mm³ An aplastic or hypoplastic reaction of the bone marrow to the sulpha therapy was suspected It was therefore thought wise to withdraw sulpha After about 2 weeks the number of white blood cells reticulocytes and thrombocytes again began to increase Sulpha therapy was resumed on May 19 1962 As before the patient s temperature fluctuated with peaks of 39 C Between April 4 and April 30 the lung lesions regressed considerably (fig 1 C and 1 D) but the patient s general condition continued to deteriorate Lumbar puncture revealed marked pleocytosis with 441 mononuclear and 408 polymorphonuclear cells per mm³ Culture of C S F gave no growth of Nocardia nor did culture of the urine despite continuous pyuria The cerebral confusion progressed the frequency of seizures increased and after a period of continuous loss of consciousness the patient died on June 19 1962

Necropsy

The apex of the left lung contained a number of hazelnut-sized cavities with grey yellow partly shiny walls In the lateral and medial part of the lower lobe were two firm well defined foci 1—2 cm in diameter with a yellow dry cut surface The lower lobe of the right lung contained a subpleural lesion half the size of a pin s head but no other focal changes

Fibrous adhesions were seen laterally and apically in the left pleura and dorsally in the right pleura.

The cortex of the kidneys showed yellow white sharply defined radial streaks The left kidney also showed a grey red crumbling band about the thickness of a finger extending from the perpelvic fatty tissue to the

After repeated subculturing or more than 5 days' incubation, the microscopic picture was dominated more and more by short rods, sometimes almost coccoid forms Subculturing in liquid medium, however, in 1—2 days gave branching filaments

The usual methods of concentration of tubercle bacilli with 4 % sodium hydroxide at room temperature for 10 minutes killed all the Nocardiae Table I shows the results after treatment of a suspension of a pure culture of the strain with an equal volume of either 6 % sulphuric acid or 4 % sodium hydroxide for 1, 3, 5 or 10 minutes at room temperature

The results of a susceptibility test on blood agar with the disc diffusion method (10, 11) are given in table II

Methods for grouping and typing of mycobacteria (17, 18, 19) gave the following results

Salicylate effect test *negative*, less than 30 % increase of oxygen consumption

Catalase activity test *positive*, value more than 150

Susceptibility determination *resistant* to 100 mcg INH/ml, 25 mcg thenoyl 2 hydrazine/ml

Niacin test *negative*

Guinea pig virulence test *negative* for 0 1 as well as 1 0 mg bacterial mass injected subcutaneously

Transhydrogenase activity methylene blue completely decolorized within 60 minutes

Pigment formation on Lowenstein-Jensen-medium negative photochromogenesis test but in continuous light pale rose pigment after 10 days The chrome yellow smooth variant formed pigment in the dark as well as in the light

The physiological properties of the strain were examined with Gordon and Mihm's technique (13, 14) and compared with known strains of Nocardia The results are given in table III where the percentage distributions of the positive results for different species of Nocardia according to Gordon and Mihm are also included It is clear from the table that the organism isolated may, according to Gordon and Mihm, be regarded as identical with Nocardia asteroides The two variants of colonies resembled one another regarding all of the 23 most important reactions given by Gordon and Mihm but differed in certain respects in other reactions

Treatment was concentrated mainly on the nocardiosis The administration of steroids was reduced, as it was suspected that the treatment might have affected the course of the infection Complete withdrawal of steroids was contraindicated by the patient s haemolytic anaemia The further course and results of treatment are given in fig 2

Initially the patient was treated with tuberculostatics, for the possibility of a fulminant tuberculosis could not be definitely excluded at that time On March 8 treatment with massive doses of benzyl penicillin was started according to a dosage schema given by Pellegrino and Henderson (32) During this treatment the patient's general condition improved, her temperature returned to normal, and the sputum volume decreased However, X ray (fig 1 B) showed a progress of the pulmonary lesions and the occurrence of pleural effusions on both sides About March, 22 the patient became worse with high grade fever At that time sulfa therapy (Sulfacombin ' 8 g/day — a combination of sulphadiazine sulphamidine, and sulphamerazine) was started A subsequent new bout of high fever was accompanied by generalized seizures The attacks were not followed by any neurological sequelae No stiffness of the neck or papilloedema could be demonstrated After this period the patient's sensorium was periodically more or less affected Owing to increased stagnation of secretion in the respiratory tract respiratory insufficiency occurred, and on April 4 the patient was transferred to the department for infectious diseases where tracheotomy was done and artificial respiration was instituted

During continuous sulpha treatment the pulmonary lesions regressed (see chest X ray fig 1 D) and repeated cultures from bronchial secretions no longer were positive for Nocardia

Despite repeated blood transfusions the blood values fell Also the number of white blood cells decreased to 2 000/mm³ from formerly 8 000—12,000/mm³ The number of

circumstances are cryptococcosis, sporotrichosis and nocardiosis Nocardiosis has often been reported as a complication of haematological diseases particularly leukaemia (7, 16, 24, 25) Withmore et al (41) have described a case of nocardiosis in acquired haemolytic anaemia in a patient who had been receiving steroid treatment and in whom the development of that infection resembled that in our case

Lowering of the general resistance owing to anaemia neutropenia or serum protein disturbances, all of which are common in haematological disorders is probably sufficient to favour such infections Steroid treatment especially if long, may also predispose to these infections 8 12 22 25 36) A curious tendency to develop nocardiosis has been reported in some cases of pulmonary alveolar proteinosis (2 6)

Our patient was in a fairly good general condition until January 1962 She had had periods of anaemia but no neutropenia or serum protein disturbances The rapid deterioration coincided with the pulmonary complication, which occurred in association with an increase of the steroid dose Steroid treatment in this case was clearly warranted by the haemolytic anaemia

In view of the increasing frequency of mycosis it is important for the clinician to be acquainted with this type of disease and to bear in mind that it is prone to complicate a number of diseases liable to become chronic particularly if the patients have been treated for a long time with steroids The possibility of a correct diagnosis in a given case is low unless the clinician informs the bacteriologist that mycosis is suspected Special methods for examination can then be used Since Nocardia, like anonymous mycobacteria, may occur in the sputum in the absence of disease, it is apparent from this case that repeated demonstration of the causal agent is necessary for the diagnosis of a Nocardia infection The samples should be taken as close to the lesions as possible to get representative material In our case a pure culture of Nocardia asteroides could be demonstrated in material obtained by bronchoscopy This sampling procedure has been reported to be successful also in other cases (5 24)

The culture is as a rule read after 24—48 hours incubation, by which time gross colonies of Nocardia are usually not recognized Culture on ordinary media involves the risk of partial or complete overgrowth of Nocardia colonies by rapidly growing bacteria e g coliform If the clinician requests culture for fungi the selective media used often are inhibitory even to Nocardia If tuberculosis is suspected the sample is as a rule treated with sulphuric acid or sodium hydroxide, which kills the Nocardia Thus in the event of obscure pulmonary lesions the bacteriologist must be informed that the sample should be examined for ordinary bacteria and fungi as well as for mycobacteria and Nocardia

Of the media used WAP medium proved best since it also partly inhibited the Gram negative flora but allowed Nocardia to grow with abundant aerial mycelium and distinct pigmentation Blood agar likewise gave good growth of Nocardia but when coliform bacteria also occurred they overgrew many of the Nocardia colonies



Fig 3 Filamentous microorganisms of Nocardia asteroides and macrophages in a section from a perinephritic abscess Staining according to Grocott (27)

surface of the kidney and continuing laterally backwards to the perirenal fatty tissue with a golfball sized abscess with viscous green pus Similar abscesses were observed in the right groin and just above the right knee (without any connection with the joint cavity) None of these abscesses were in connection with osseous changes

The dura and arachnoid were tense The brain was swollen with compression grooves on the tonsils of the cerebellum In all of the lobes of the cerebrum and in the right hemisphere of the cerebellum were pea to golfball sized foci with firm margins enclosing viscous, green material In both occipital lobes the foci were surrounded by fairly liquefied tissue The left lateral ventricle contained cloudy fluid Punctate haemorrhages were seen in the pons

Below the entrance of the renal veins the inferior vena cava was filled with masses of green grey thrombi with dry cut surfaces These thrombi continued into the vv iliacae communes, vv iliacae internae and vv iliacae externae and both femoral veins Thrombi were also found in the right renal vein and in the left jugular vein

Tracheostoma Decubitus over sacrum and right thigh

Microscopical examination Bone marrow normal blood forming marrow in vertebral bodies, active blood formation in femoral marrow Spleen (580 g) fairly numerous erythropoietic cells

Siderosis of lungs, spleen and bone marrow

The lower lobe of the left lung showed foci of caseous necrotic tissue with small calcifications, a fibrous capsule and a few multinuclear giant cells of Langhan s type. In the surrounding tissue were foci with caseous necrotic foci surrounded by an epitheloid cell brim with polynuclear giant cells. The apex of the left lung showed cavities bordered by histiocytes and in part by epitheloid cell ramparts, and a tendency to caseous necrosis towards the lumen A number of cavities had fibrous walls with a smooth inner side. In other parts were small areas of disintegration filled with leucocytes The surrounding lung parenchyma was undergoing fibrosis and showed numerous phagocytes, some containing brown pigment

Lymph nodes from the hilus of the lung showed no necrosis or granuloma

Abscesses in the brain, in the neck and in the left kidney as well as in the right groin and the right knee showed the same picture a wall of richly vascularised granulation tissue with massive deposition of histiocytes leucocytes and plasma cells as well as polynuclear cells of varying appearance

Staining according to Grocott showed thread like elements resembling the hyphae of Nocardia (fig 3) in the perinephritic abscesses

Discussion

Infections of the oral cavity, throat and genital tract with fungi — the most important belonging to the Candida group — is a common complication in patients with a poor general condition treated for a long time with antibiotics, steroids or cytostatics Infections with Candida can be treated rather effectively with various mycostatics But disseminated infections with these fungi are sometimes very difficult to control (1, 3, 33, 35) Other infections prone to occur under such

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In the culture technique used by Gordon and Mihm the different stages — preparation of the media, culturing of the strains and reading of the results — are all very time-consuming. Therefore extensive endeavours were made to find a method for distinguishing Nocardia-species by their amidase pattern (21). One of the findings was that a change in the composition of Bönicke's series of amides (4) gave typical amidase patterns for the available strains of both N. asteroides and N. caviae. The isolated organism also showed an amidase pattern identical with that of the other N. asteroides strains.

In addition, unlike all the Nocardia strains, the rapidly growing mycobacteria M. smegmatis, M. phlei and M. fortuitum were all able to break down formamide, as did M. rhodochrous. It therefore appears that examination of the amidase patterns of Nocardiae might be a simple way of distinguishing different species of Nocardia not only from one another but also from the rapidly growing mycobacteria.

Infections with Nocardia are characterised by their therapeutic refractoriness. Several authors claim that sulpha therapy produces the best effect in nocardiosis (31, 37, 38). In our case the strain proved sensitive to sulpha in a concentration of 0.1 mg/ml. The reason why we initially refrained from giving sulpha therapy was that we feared such treatment might have an adverse effect on the patient's blood disease. This fear proved justified during a later phase of treatment with sulpha which was followed by marrow depression. The bone marrow recovered part of its activity on withdrawal of sulpha. The striking improvement in the condition of the lungs after sulpha therapy was surely due to a large extent to very careful drainage of secretion from the respiratory tracts by the tracheotomy. Strangely enough, the infection appeared to spread during the period that the lung affection improved. Culture of necropsy specimens of the lung, liver and spleen gave no growth, but growth was obtained from an abscess in the brain and from another in one of the kidneys. Since nocardiosis has a marked tendency to produce circumscribed infectious foci with or without abscess formation (metastases), antibiotics and chemotherapeutics are hardly able to exert any effect even when given in large doses. In such cases surgical drainage and sulpha therapy in combination are justified (25, 34).

Summary

A report is given of a case of acquired haemolytic anaemia treated with steroids. Temporarily there was good haematological remission but after about 6 months pulmonary changes due to Nocardia asteroides supervened. During sulpha therapy and careful drainage of secretion from the respiratory tract via a tracheotomy and periods of artificial respiration, a striking improvement of the pulmonary changes was observed. But the infection spread to the kidneys and brain and the outcome was fatal. Factors favouring infection with Nocardia and fungi are mentioned and the diagnosis and treatment of the disease are discussed.

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The Estimation with Radioactive Colloidal Gold of Liver Blood Flow in Cirrhotic Patients

By

L Kallai D Ivančević, A Hahn, N Hadžić S Knežević and I Šimonović

The fundamental investigations of Dobson and Jones (10) have shown that the clearance of radioactive colloid from the blood may usefully serve for the estimation of liver blood flow Vetter et al (17) applied radioactive colloidal gold (Au 198) to this end, and this simple method causing no discomfort to the patient, has been used in clinical examinations since There is however no full agreement on the method of measurement to adopt in addition to the measurement of the concentration of radio colloids in the blood external measurements over the liver or some other parts of the body are also performed Examinations with radiocolloids have afforded insight into hepatic circulation and have proved of special interest in patients with liver cirrhosis The results obtained by different authors however show certain discrepancies 1 2 3 13 16 17)

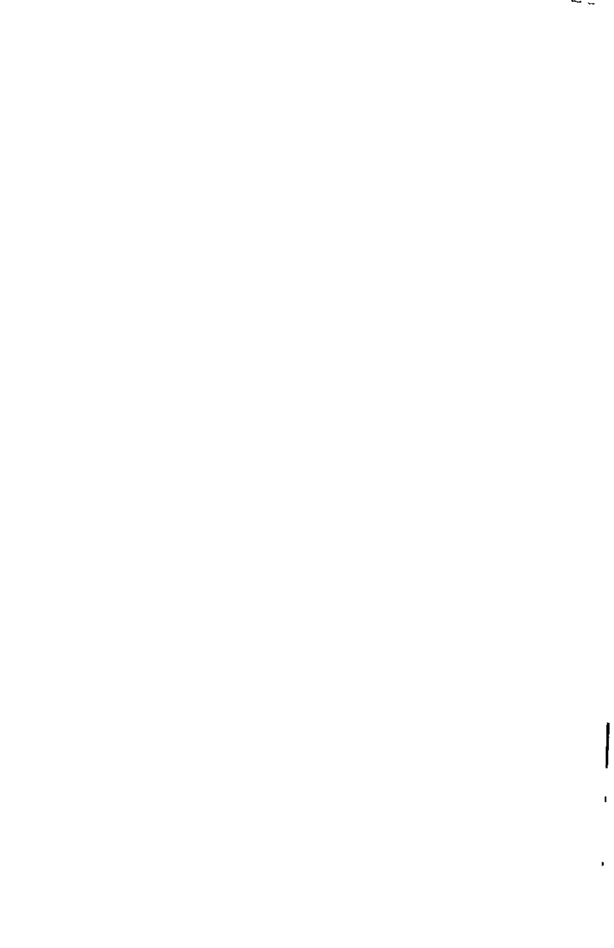
The scope of the present study was to establish the method most convenient for the assessment of hepatic circulation, by means of colloidal radio-gold We also wished to test by the application of our own method and a standard preparation of colloidal gold the fractional clearance of colloid and the liver blood flow in patients with liver cirrhosis and in controls

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Material and methods

A total of 27 patients with liver cirrhosis were examined 19 subjects were male 8 female The diagnosis was established on the basis of detailed clinical examinations and biochemical findings confirmed by laparoscopy or liver biopsy All patients were hospitalised under standard hospital conditions There were 13 compensated and 14 decompensated cases of cirrhosis In order to establish normal values 19 controls were also examined of which 8 were male and 11 female

Radioactive colloidal gold of French production (Saclay Au —198— 8—3) with a declared mean size of particles of about 300 A was used The original preparation was not diluted A quantity of 50 to 100 μc was given by intravenous injection in the recumbent patient Blood samples were recovered from the vein in the contralateral arm In the beginning we used to collect several blood



samples The hypothesis that y x, where x results obtained by the blood sampling method and y results obtained by the surface counting method, was tested by fitting a straight line $y = a + bx$ to the data by the method of least squares (18) It was found in both cases that neither the intercept a was significantly different from zero, nor the slope b significantly different from 1 This showed that a straight line through the origin with a slope of unity fits the data within the experimental error

According to our findings the good correlation of results yielded by external measurements of liver and thigh activity with those of measurements of blood activity seems to justify the use of external measurements by themselves without recourse to the collection of blood samples

A slow component is not encountered in the analysis of the liver activity curve probably because the liver retains mainly the larger particles of colloid This finding is in agreement with the observation made by Bruce Torrance and Govenlock 6)

In analysing measurements of thigh activity we consider it important not to calculate on the basis of the real values for thigh activity but rather to diminish them by the value obtained during the equilibrium phase In this manner we eliminate at least in part the effect produced by the activity collected in the bone marrow upon the total thigh activity curve

The data obtained by means of the scaler are more exact and analysis thereof is more dependable than are the results yielded by rate meter and recorder The use of the latter is endangered by the difficulty of selecting the proper scale — thus making the analysis of curves more difficult

While introducing the method we tried to make use of external measurements of the head and heart, but have abandoned it since Although measurements of head activity did show a brief mixing period, the activity was relatively low In heart measurements it is difficult to find a satisfactory solution to the problem of collimation towards the organs lying close by the liver and spleen

In the analysis and interpretation of the results, fractional clearance was considered a biologically more important and essential datum than the calculated estimated liver blood flow Fractional clearance (2) offers data on the fraction of the total blood volume cleared by the liver in the course of a minute On the basis of this the part of the blood volume passing through the liver per minute is calculated The setting up of these conclusions includes an error due to the differences of colloid extraction in the liver (2 5 11) The estimated liver blood flow yields absolute values of the quantity of blood flowing through the liver per minute (absolute clearance (2)) This however includes the possibility of new errors (as e g blood volume)

Table I and fig 3 show the values of fractional clearance in our groups of controls and compensated and decompensated cirrhotics

Merely in order to give more plasticity to the results and aware of possible errors (especially in cirrhoses) we also calculated the values of estimated (minimal) blood flow through the liver The blood

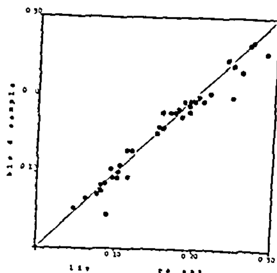


Fig 1 Comparison of values of fractional clearance estimated simultaneously by means of blood sampling and external measurement of the liver

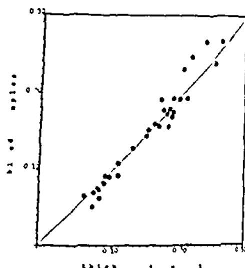


Fig 2 Comparison of values of fractional clearance estimated simultaneously by blood sampling and external measurement of thigh

samples in the course of the first 60 minutes, later we limited these to five samples taken between the second and fifteenth minute Analysis of blood activity curves was performed according to the classical method of calculating the rate constants of the disappearance of colloid from the blood (1,10 17) Only the early phase of the disappearance curve was considered it represents an almost pure rapid component Thigh activity was measured by means of a scintillation counter with a cylindrical collimator the multiple G M counter consisting of 4 G M tubes connected in parallel positioned and fixed over the liver region used to measure liver activity At first, recordings of the activity of liver and thigh were taken by rate meter and recorder which were later replaced by scalers Measurement was begun every 30 seconds and had a duration of 20 seconds each External measurements were concluded only after achieving equilibrium over both the liver and thigh, but not before the thirtieth minute The values obtained by measuring liver activity were subtracted from the values obtained in the stage of equilibrium The values obtained in the stage of equilibrium were subtracted from the values resulting from measurement of thigh activity

The data obtained by measurement of blood samples and external measurements served as basis for the calculation of fractional clearance By multiplying the disappearance rate constant (K) (fractional clearance) with the blood volume we calculated the estimated (minimal) liver blood flow The blood volume was estimated according to the nomograms of Hicks et al (12)

Results and discussion

In the first part of our study the results obtained by measurement of blood samples were compared with the results of external measurements of liver and thigh The values of disappearance rate constant (K values, fractional clearance) obtained in the same patient by means of measurement of blood samples and of liver activity are presented in fig 1 Fig 2 shows the values of disappearance rate constant obtained by measurement of the activity in blood and thigh The external measurements are seen to correlate well with the measurements of blood

icant difference between the group of decompensated and that of compensated cirrhoses (p 0 01)

The values of estimated liver blood flow are also much lower in the group of cirrhoses (table II)

Our results allow for the confirmation of the results of other authors (3, 8, 13, 15, 17) who found that values for the fractional clearance of colloidal radiogold and the estimated liver blood flow were reduced in cirrhoses of the liver The decrease is greater in decompensated cirrhoses than in compensated ones For this reason we trust that the technique using radioactive colloidal gold may not only be of help in the assessment of blood circulation in liver cirrhoses but may permit the forming of conclusions on the gravity of the disease

Conclusions and summary

Methods of measurement of hepatic circulation by means of clearance of intravenously injected colloidal radiogold of particle size 300 A were investigated The disappearance rate of colloid from the blood was estimated by means of blood samples and external measurements of liver and thigh A good correlation of results was obtained which may warrant the replacement of the blood sampling method by the external measurement methods

The fractional clearance of colloidal gold and the estimated liver blood flow were investigated in 19 controls 13 patients with compensated cirrhosis and 14 patients with decompensated cirrhosis of the liver Either group of cirrhosis shows a statistically significant reduction of fractional clearance and of the estimated liver blood flow These values are significantly reduced in patients with decompensated cirrhosis as compared with the compensated *ones*

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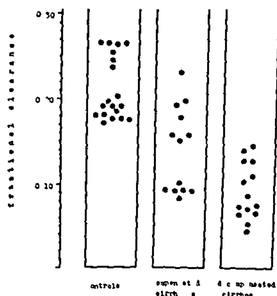


Fig 3 Results of measurement of fractional clearance by blood sampling in the group of controls, of compensated and of decompensated liver cirrhoses

volume was not measured directly, we also estimated it on the basis of the nomograms of Hicks (12) as have Bruce Torrance and Gowenlock (6). The results obtained in the group of controls and cirrhoses are summarized in table II.

Our results in normal values of fractional clearance rates correspond to those of Vetter et al (17), and of Baptista and Silva Carvalho (1). The normal values obtained by Dobson et al (10), and Nardi and Palazzi (15) by means of radioactive colloidal chromiumphosphate are somewhat higher (0.282 and 0.335 respectively) which is understandable in view of the considerably larger particle size of chromium phosphate, the case is similar in labelled heat-denatured albumin (4, 7, 8, 9). Krook (13) and Benhamou (2) obtained lower values by using radiogold (0.158 and 0.169 respectively). An explanation could be found in differences in the preparation of colloidal gold and in the quantity of gelatin added which stabilizes the solution but affects the fractional clearance of colloid (14).

TABLE I Statistical evaluation of results of fractional clearance (blood sampling method) in the group of controls (A) of compensated (B) and of decompensated (C) liver cirrhoses

	A	B	C
No of cases	19	13	14
Mean	0.213	0.140	0.91
S D	0.036	0.048	0.032
S D of mean	0.00835	0.0134	0.00855

t test A/B t = 4.63 p 0.001
t test A/C t = 10.11 p 0.001
t test B/C t = 3.03 p 0.01

TABLE II Statistical evaluation of results of estimated liver blood flow (blood sampling method) in the group of controls and of liver cirrhoses

	Controls	Cirrhoses
No of cases	19	[illegible]
Mean	919.42 ml	53[illegible] ml
S D	208 ml	229 ml
S D of mean	47.7 ml	44.1 ml

t test t = 59.5 p 0.001

As seen on table I and fig 3, values for fractional clearance in the group of compensated cirrhoses and the group of decompensated cirrhoses differ from those in the group of controls. The difference between the group of compensated cirrhoses and the controls is statistically significant (p 0.001), as is the difference between decompensated cirrhoses and controls (p 0.001). There is even a signif-

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On the Relationship Between Water Hardness and Death Rate in Cardiovascular Diseases[1]

By

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The marked geographic variation in the distribution of cardiovascular diseases is well known and often emphasized (4 10 11) It has been the impetus to many studies on the possible influence of various environmental factors One of these factors is the chemical composition of drinking water Thus epidemiologic *investigations in* Japan, the United *States and Great Britain have indicated* a higher death rate from cerebrovascular and cardiovascular diseases in areas with soft water than in hard water regions (1 3, 5 7 9 12 13 14) High sulphate concentration in the river water in Japan (5 12) has also been positively correlated to death rate in cerebrovascular disease In some studies, *however no correlation has been found* 6 8) The matter has been discussed by Schroeder (15) Dingle et al (2) and others Gastric cancer has been found more commonly in areas where the water is soft and has a low pH (16) In the present paper we will briefly report on results obtained from Sweden

Submitted for publication February 23 1965

Material

The study is based on material from all Swedish towns with more than 25 000 inhabitants, 33 in number and in addition Visby on the island of Gotland The distribution *within the country is seen in fig* 1 in which also areas with primitive rocks (white) and sedimentary rocks (shaded) are indicated *In table I* information is given for each town about number of inhabitants geology type of water source and some chemical parameters in drinking water as supplied

From the local water works we have obtained the available annual mean values for 23 chemical analyses of the finished drinking water supply from 1940 to 1962 Sixteen of these analyses on at least 1 sample a year are required by law for all works The period thus covered varies from 5 to 24 years (mean 16) the number of samples subjected to routine analyses from 1 to about 50 a year *Some parameters* have been excluded because the values are near or below the limit of the analytical method or because they have been determined at too few works The remaining water parameters are listed in table II

[1] Read before the European Congress of Cardiology in Prague August 1964

TABLE II The water parameters studied, their mean values and the number of towns from which the respective data were obtained The water hardness is given in German degrees dH, which is mg CaO/100 ml 1 dH° corresponds to about 7 2 mg Ca/l

Water parameter	Range and mean values	No of towns
Complex expressions		
MnO_4 —consumption	3 5—11—36 mg/l	34
Total solids	64—210—790 mg/l	32
Ignition residue	41—180—740 mg/l	33
Total water hardness	1 6—6 5—19 mg CaO/100 ml	34
Marble attacking H_2SO_4	0—4 2—22 mg/l	33
Cations		
Ca^{2+}	11—39—190 mg/l	29
Mg^{2+}	2 5—9 8—61 mg/l	14
pH (H^+)	6 5—7 5—8 4	34
Anions		
HCO_3^-	15—100—400 mg/l	34
SO_4^{2-}	5 4—35—130 mg/l	34
Cl^-	5 6—27—120 mg/l	34
SiO_2	1 0—6 4—21 mg/l	16
F^-	0 10—0 41—1 5 mg/l	25

From Statistiska Centralbyrån Stockholm we have obtained the number of inhabitants in the censuses 1950 and 1960 the absolute number of deaths from 19 specific groups of causes and from all causes for both sexes and the age groups 25—44 45—64 and 65—74 years separately for each year during the period 1951—60 in the respective towns and the mean age adjusted death rate for the period The groups of diagnoses are given in table III

Methods

1 Correlations between the various water parameters, and between death rates from various causes and each of the water parameters were calculated with the aid of a computer
2 For the age group 25—74 years the death rates from causes 420 and 422 were plotted separately as well as jointly versus total water hardness and concentration of Ca^{2+} The equations of the corresponding regression lines and the significance of the slope (t-coefficients) were calculated
3 For the age groups and sexes separately multiple regression analyses of the death rate from causes 420 and 422 versus six water parameters which were considered to have little interdependence were made in unselected order with a computer
4 The ratios of death rates from causes 420/422 were calculated for the 3 largest cities for each year during the period from the absolute number of deaths in the age group 25—74 years The same ratios were also calculated for 9 towns with a mean water hardness less than 3 mg CaO/100 ml and 6 towns with more than 14 mg CaO/100 ml
5 The mean death rates from cause 422 were calculated for the periods 1950—55 and 1956—60 and correlated to the mean

TABLE I Number of inhabitants, characteristics of geology and water source, and mean values of total water hardness and concentration of Ca^{++} for each town in the study

Towns	No of inhabitants (1/1 1961)	Increase 1950—60 (%)	Ground	Vegetation	Type of water source	Total water hardness	Conc Ca (mg/l)
Stockholm	808,294	8 4	Prim	Agr mead dec con rocks	Lake	5 0	98
Goteborg	405,252	15	Prim	Agr mead dec. con rocks	Lake river, art. subsoil	2 1	1[illegible]
Malmo	229,248	20	Chalc	Agr mead dec	Nat art subsoil	17	1[illegible]0
Norrkoping	90,800	7 4	Prim	Agr dec con	River	4 1	[illegible]
Halsingborg	76,541	7 0	Trias		Nat art subsoil	15	
Uppsala	77,529	23	Prim		Subsoil	14	100
Västerås	77,869	30	Prim	Agr mead dec. con rocks	Art subsoil	7 6	34
Örebro	75,527	13	Cambr	Mead con	Art subsoil	4 5	3[illegible]
Borås	66,930	15	Prim	Con rocks	Lake	3 7	[illegible]6
Linkoping	65,198	20	Prim / Cambr	Agr mead	River	3 6	26
Eskilstuna	59,016	11	Prim	Agr mead dec con rocks	Nat art subsoil	5 1	
Gävle	54 770	17	Sed		Nat art subsoil	9 4	69
Jonkoping	50 626	14	Sed	Agr mead dec con rocks	Lake spring subsoil	3 2	23
Solna	50,969	36	Prim	Agr mead dec con rocks	Lake	6 0	31
Karlstad	43 033	21	Prim	Con	River, art subsoil	1 8	12
Lund	40 405	19	Chalc	Mead	Lake subsoil	16	1[illegible]0
Halmstad	39 051	11	Prim	Agr	Subsoil	6 0	43
Karlskoga	35 456	13	Prim	Agr	Nat art subsoil	5 1	36
Uddevalla	34 260	37	Prim	Con rocks	Lake	2 4	17
Karlskrona	32 973	5 6	Prim	Agr mead dec con rocks	River	2 9	20
Sodertalje	33 171	31	Prim	Agr mead con	Nat art subsoil	4 5	32
Trollhättan	31 937	32	Prim	Agr mead dec con rocks	River	1 8	13
Kalmar	30,791	14	Cambr	Agr con	Nat art subsoil	3 5	25
Luleå	30 566	35	Prim	Con	Nat art subsoil	4 1	19
Sundsvall	29 419	14	Prim	Agr mead con	Lake subsoil	1 8	
Landskrona	28 864	15	Chalc	Agr mead	Lake river spring	19	
Lidingo	29 424	44	Prim	Agr mead dec con rocks	Lake	5 0	28
Sundbyberg	27,068	10	Prim	Agr mead dec con rocks	Lake	6 0	31
Motala	27 174	10	Cambr	Mead dec	Lake	2 0	14
Kiruna	26 748	40	Prim			2 8	20
Borlänge	26 479	22	Prim	Agr con		7 1	51
Kristianstad	25 804	7 3	Chalc	Agr	Subsoil	12	87
Molndal	26 428	27	Prim			1 6	11
Visby	15 604	8 3	Cambr			14	

Prim* = primitive rocks sed = sedimentary rocks cambr = cambrion silurian, trias = trias jurassic, agr = agricultural mead = meadows dec = deciduous, con = coniferous, nat. = natural, art = artificial subsoil water (the water is pumped up twice and perfused through a gravel ridge)

TABLE III Groups of causes of death The code numbers are given according to the international list of diagnosis. The present study has been devoted mainly to the groups in the frame

Code	Cause
1—8	Tuberculosis
140—150	Malignant tumours of mouth pharynx and oesophagus
151	Malignant gastric tumours
152—161	Malignant tumours of the intestine liver, pancreas peritoneum larynx nose with sinuses and middle ear
162—163	Malignant tumours of trachea bronchi and lungs
164—165	Malignant tumours of mediastinum metastatic tumours in the thorax
170	Malignant tumours of the breast
171—205	Malignant tumours of the urogenital organs, malignant melanoma and other tumours of the integument malignant tumours of the eyes central nervous system thyroid and other endocrine glands bone connective tissue, muscles lymph glands and blood forming organs
330—334	Vascular disease of the central nervous system
400 416	Rheumatic fever and chronic rheumatic heart disease
420	Arteriosclerotic heart disease
421	Chronic non rheumatic endocarditis
422	Other degenerative heart diseases
430 —447	Other endo-myo-pericarditis functional heart disease arterial hypertension
450—468	Arterial and venous diseases embolia trombosis arterial hypotension diseases of lymphatic glands and vessels
470 527	Acute infection of the upper respiratory system influenza pneumonia bronchitis and other diseases of the respiratory organs
810 962	Traffic casualty poisoning and other accidents complications of treatment
963—999	Suicide murder war
	Other causes
	Total mortality

heart diseases (422) both in males and females Similar patterns were to some extent obtained in the other age groups

In the present investigation only the group of cerebrovascular and cardiovascular diseases was studied in some detail Table VII summarizes the correlation r-coefficients) to water hardness for all the sex and age groups In the youngest male group a highly significant negative correlation ($p < 0.005$ for water hardness was found with other degenerative heart diseases (422) mainly so-called cardiosclerosis or myocardial degeneration A significantly negative correlation ($p < 0.01$) was found for cerebrovascular diseases in the middle age group of women In addition, scattered significances at the $p < 0.05$ level were seen

Table VIII shows the correlation coefficients (r) for the death rates from these cerebrovascular and cardiovascular diseases to the concentration of Ca^{+} For males a highly significant negative correlation was found to the group of "other degenerative heart diseases (422) in all age groups ($p < 0.005$) In the

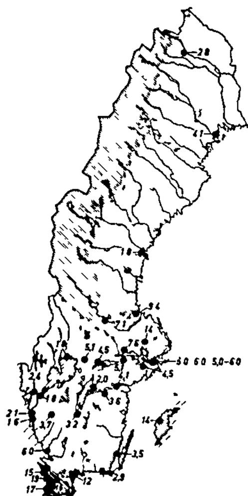


Fig 1 Map of Sweden indicating the distribution and the mean values of total water hardness of the towns dealt with in the present study White areas correspond to primitive rocks and shaded areas to sedimentary rocks

concentration of Ca^{2+} for the whole period The 10 year mean number of inhabitants was, an approximation, taken to be the value for 1955

6 The death rates from causes 420 and 422 in Stockholm for each year were calculated after interpolation of the number of inhabitants between censuses undertaken in 1950, 1955 and 1960

In the tables the following symbols for the degree of significance will be used * = probably significant, $p < 0.05$, ** = significant, $p < 0.01$, *** = highly significant $p < 0.005$

Results

The correlation between the various water parameters is indicated in table IV Evidently, there is a large group of water parameters varying together total solids, ignition residue, total water hardness, concentrations of Mg^{2+}, HCO_3^-, SO_4^{2-}, Cl^-, SiO_2, and F^- The concentration of Ca^{2+} is correlated to total water hardness and concentration of HCO_3^- but not significantly related to other factors, MnO_4^--consumption, an expression of the amount of reducing substances (mainly organic) in the water, tends to vary inversely with the large group of inorganic constituents mentioned above The pH is significantly correlated only to the concentration of marble-attacking H_2SO_3

The results of the correlation between death rates and water parameters (r coefficients) are summarized in table V (males) and table VI (females) Total mortality — death rates from causes not here specified as well as from most of the specified causes — were not significantly related to any of the water parameters For certain groups of causes, however, e g malignant tumours of the upper digestive tract (140—150), vascular diseases (450—468) in the males, and breast cancer (170) in the females, highly significant positive correlations to the inorganic group of water parameters were found ($p < 0.005$) For death rate from cerebrovascular diseases in the females, a probably significant negative correlation was found to the same group of water parameters ($p < 0.05$) The concentration of Ca^{2+} was significantly negatively correlated to the death rate from 'other degenerative

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Code	Cause
1—8	Tuberculosis
140—150	Malignant tumours of mouth pharynx and oesophagus
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152—161	Malignant tumours of the intestine, liver, pancreas peritoneum larynx nose with sinuses and middle ear
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164—165	Malignant tumours of *mediastinum* metastatic tumours in the thorax
170	Malignant tumours of the breast
171—205	Malignant tumours of the urogenital organs malignant melanoma and other tumours of the integument malignant tumours of the eyes, central nervous system *thyroid and* other endocrine glands bone connective tissue muscles lymph glands and blood forming organs
330—334	Vascular disease of the central nervous system
400 - 416	Rheumatic fever and chronic rheumatic heart disease
420	Arteriosclerotic heart disease
421	Chronic non rheumatic endocarditis
422	Other degenerative heart diseases
430—447	Other endo-myo-pericarditis functional heart disease arterial hypertension
450—468	Arterial and venous diseases embolia, trombosis arterial hypotension diseases of lymphatic glands and vessels
470—527	Acute infection of the upper respiratory system influenza pneumonia bronchitis, and *other diseases of the respiratory organs*
810 962	Traffic casualty poisoning and other accidents complications of treatment
963—999	Suicide murder war
	Other causes
	Total mortality

heart diseases (422) both in males and females Similar patterns were to some extent obtained in the other age groups

In the present investigation only the group of cerebrovascular and cardiovascular diseases was studied in some detail Table VII summarizes the correlation r-coefficients) to water hardness for all the sex and age groups In the youngest male group a highly significant negative correlation ($p < 0.005$ for water hardness was found with "other degenerative heart diseases 422) mainly so-called "cardiosclerosis or "myocardial degeneration A significantly negative correlation ($p < 0.01$) was found for cerebrovascular diseases in the middle age group of women In addition scattered significances at the $p < 0.05$ level were seen

Table VIII shows the correlation coefficients (*r*) *for the death rates from* these cerebrovascular and cardiovascular diseases to the concentration of Ca[2] For males a highly significant negative correlation was found to the group of "other degenerative heart diseases (422) in all age groups ($p < 0.005$) In the

TABLE IV Correlation coefficients (r) between the chemical parameters in the drinking water as

	MnO_4^- consumption	Total solids	Ignition residue	Total water hardness	Ca^{++}
Total solids	−0 297*				
Ignition residue	−0 317*	+0 976***			
Water hardness	−0 395*	+0 886***	+0 931***		
Ca^{++}	−0 238	+0 281	+0 237	+0 394*	
Mg^{++}	−0 092	+0 682***	+0 670***	+0 486*	−0 080
pH	−0 023	+0 030	+0 040	+0 066	+0 130
HCO_3^-	−0 384*	+0 847***	+0 905***	+0 978***	+0 335*
CO_2	−0 056	−0 108	−0 108	−0 165	−0 104
SO_4^{2-}	−0 062	+0 762***	+0 740***	+0 666***	+0 064
Cl^-	−0 185	+0 814***	+0 790***	+0 579***	+0 113
SiO_2	+0 067	+0 576**	+0 553*	+0 459*	+0 146
I^-	−0 139	+0 496**	+0 516***	+0 438*	−0 001

TABLE V Correlation coefficients (r) for death rate from all causes against all water parameters

Code No	MnO_4 consumption	Total solids	Ignition residue	Water hardness	Ca^{++}	Mg^+
1−8	−0 013	+0 037	+0 055	+0 066	+0 026	−0 434
140−150	−0 101	+0 564***	+0 547***	+0 554***	+0 357*	+0 582*
151	+0 055	+0 005	−0 063	−0 194	−0 155	+0 061
152−161	+0 349*	+0 080	+0 078	+0 056	+0 072	+0 307
162−163	−0 088	+0 120	+0 147	+0 165	+0 137	−0 169
164−165	−0 152	−0 169	−0 193	−0 139	−0 063	−0 150
170	+0 601***	−0 006	−0 030	−0 013	+0 030	−0 011
171−205	+0 212	+0 103	+0 173	+0 098	+0 073	+0 383
330−334	+0 310*	−0 225	−0 160	−0 189	−0 258	−0 358
400−416	+0 030	+0 086	+0 021	+0 020	+0 198	−0 181
420	+0 069	−0 046	−0 093	−0 157	+0 004	−0 095
421	−0 221	−0 015	−0 056	−0 014	+0 092	+0 077
422	+0 154	−0 121	−0 122	−0 259	−0 486***	+0 034
430−447	+0 158	+0 232	+0 283	+0 238	−0 019	+0 450
450−468	−0 202	+0 369*	+0 361*	+0 435***	+0 470***	+0 208
470−527	+0 068	+0 034	−0 009	+0 035	+0 340*	−0 235
810−962	+0 042	−0 039	−0 097	−0 234	−0 191	+0 083
963−999	−0 082	+0 409**	+0 404**	+0 382*	+0 217	+0 280
Other causes	−0 001	+0 109	+0 148	+0 192	+0 043	+0 428
Total mortality	+0 216	+0 155	+0 146	+0 058	+0 021	+0 154

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Mg	pH	HCO_3	CO_2	SO_4	Cl	SO_2
+0 123						
−0 525*	+0 012					
0 157	0 741***	0 182				
0 672 *	+0 159	+0 608 *	−0 060			
0 548*	0 042	+0 524 **	−0 007	+0 498***		
0 715 *	0 021	+0 458	−0 079	+0 530*	+0 452*	
0 652*	0 022	+0 483**	−0 088	+0 404	+0 283	−0 535*

Males 45–64 years

pH	HCO_3	CO_2	SO_4	Cl	SO_2	F
0 199	0 028	+0 106	+0 131	+0 0 0	0 339	0 2 9
0 2 6	0 533**	0 292*	+0 552***	+0 433**	+0 402	0 240
0 0 9	0 221	+0 001	0 011	+0 224	−0 014	0 082
0 262	0 0 8	0 313*	0 316*	−0 046	−0 063	−0 054
0 316	0 097	0 786	+0 316	+0 055	−0 346	−0 247
0 025	0 1 5	0 001	0 124	0 190	0 110	0 101
0 247	0 035	0 215	0 039	−0 013	0 361	0 002
0 014	0 139	0 091	0 160	+0 019	+0 417	0 153
0 008	0 208	0 065	0 207	0 032	0 145	0 249
0 717	0 035	0 191	0 144	0 019	0 040	0 068
0 76	0 149	0 136	0 120	0 110	0 063	0 068
0 234	0 044	0 273	0 142	0 234	0 084	0 174
0 125	0 249	0 024	0 051	0 096	0 2 6	0 046
0 1 5	0 275	0 78	−0 168	0 124	0 341	−0 513 *
0 310	0 417*	0 187	0 359	0 0 9	−0 398	0 165
0 403 *	0 021	0 238*	0 232	0 187	0 297	0 009
0 0 3	0 718	0 061	0 183	0 245	0 120	0 023
0 338	0 30	0 191	0 389*	0 283	0 139	0 122
0 0*	0 6	0 2 8	0 048	0 065	0 307	0 423
0 1 0	0 0 7	0 177	0 185	0 190	0 106	0 096

TABLE VI Correlation coefficients (r) for death rate from all causes against all water parameters.

Code No	MnO_4^- consumption	Total solids	Ignition residue	Water hardness	Ca^{2+}	Mg^{2+}
1–8	−0.035	−0.085	−0.153	−0.244	−0.279	−0.224
140–150	−0.159	+0.003	+0.007	+0.021	+0.171	−0.197
151	+0.096	+0.004	−0.076	−0.111	+0.063	+0.282
152–161	−0.023	−0.182	−0.057	+0.019	−0.130	−0.383
162–163	−0.008	+0.057	−0.007	+0.048	+0.250	−0.319
164–165	−0.102	−0.048	−0.051	+0.013	+0.021	−0.163
170	−0.328*	+0.405*	+0.495***	+0.481***	+0.282	+0.317
171–205	+0.106	+0.118	+0.175	+0.261	+0.142	+0.299
330–334	+0.225	−0.354*	−0.377*	−0.432**	−0.385*	−0.219
400–416	+0.152	−0.018	−0.076	−0.033	+0.186	−0.321
420	−0.078	−0.212	−0.199	−0.186	−0.025	−0.031
421	+0.378*	−0.085	−0.114	−0.187	−0.006	+0.280
422	+0.339*	−0.185	−0.232	−0.372*	−0.396*	+0.147
430–447	+0.336*	−0.250	−0.180	−0.146	−0.340*	−0.229
450–468	+0.195	+0.084	+0.044	+0.138	+0.284	+0.190
470–527	+0.091	+0.037	+0.093	+0.095	−0.025	−0.022
810–962	−0.128	−0.114	−0.140	−0.161	−0.141	−0.173
963–999	+0.103	+0.173	+0.232	+0.261	+0.108	+0.131
Other causes	+0.318*	−0.157	−0.059	−0.087	−0.252	+0.008
Total mortality	+0.326*	−0.256	−0.194	−0.204	−0.279	−0.032

females a corresponding correlation increasing with age was noticed in age group 45—64 years $p < 0.01$, and in the group 65—74 years $p < 0.005$ For none of the sex- and age groups was any correlation found between death rate from arteriosclerotic heart disease (420) and concentration of Ca^{2+}

The same relation is illustrated in figs 2 and 3 The death rates from arteriosclerotic heart disease (420) and "other degenerative heart diseases" (422) for the age group 25—74 years, both sexes together, were plotted against total water hardness and concentration of Ca^{2+}, respectively The slopes of the calculated regression lines are highly significant ($p < 0.005$) for 422 against both water parameters and significant ($p < 0.01$) for 420 against water hardness, but not against concentration of Ca^{2+}

The multiple regression analysis showed no significant correlation between any of the water parameters (MnO_4^- consumption, concentrations of Ca^{2+}, HCO_3^-, SO_4^{2-}, Cl^-, and pH) and death rate from cause 420, with the exception of concentration HCO_3^- in the youngest males The death rate from cause 422 was found to be significantly correlated to concentration of Ca^{2+} in all the male groups, and in the youngest group also to Cl^- and pH For the females the death from cause 422 was significantly correlated to pH and concentration of Ca^{2+} in

Females, 45—64 years

pH	HCO_3^-	CO_2	SO_4^{2-}	Cl	SiO_2	F^-
+0 166	−0 281	+0 030	±0 000	+0 036	−0 140	−0 305
+0 106	−0 017	−0 046	+0 036	+0 047	−0 257	−0 114
−0 060	−0 087	+0 134	+0 161	−0 216	+0 259	+0 234
−0 262	−0 025	+0 327	−0 176	−0 157	−0 020	−0 175
+0 028	−0 011	+0 077	+0 197	−0 117	−0 359	−0 137
+0 251	+0 017	−0 235	−0 045	−0 157	−0 172	+0 060
−0 038	+0 450**	+0 115	+0 370*	+0 357*	+0 148	+0 109
−0 010	+0 296*	−0 042	+0 319	−0 131	+0 142	+0 316
−0 174	−0 401**	+0 035	−0 398**	−0 132	−0 079	−0 123
+0 067	−0 080	+0 037	+0 334	−0 161	−0 415	−0 057
−0 340	−0 109	+0 303*	−0 287	−0 190	+0 171	+0 155
−0 052	−0 111	−0 013	+0 008	−0 127	+0 167	+0 040
−0 411*	−0 320*	+0 311*	−0 155	−0 133	+0 212	+0 257
−0 135	−0 111	+0 064	−0 341	−0 151	+0 079	−0 039
0 051	+0 190	−0 167	+0 041	−0 176	+0 456*	+0 135
0 481**	+0 069	−0 431**	+0 104	−0 032	−0 328	+0 180
0 007	−0 168	+0 147	−0 082	−0 079	−0 229	−0 068
0 242	+0 208	−0 030	+0 440**	+0 052	−0 196	+0 207
−0 132	−0 065	+0 054	−0 076	−0 061	−0 027	+0 197
−0 [illegible]81	−0 153	+0 235	−0 126	−0 246	+0 074	+0 224

TABLE VII Correlation coefficients (r) for death rate from different groups of cardiovascular diseases and total mortality against total water hardness

Code No	25—44 years		45—64 years		65—74 years	
	Males	Females	Males	Females	Males	Females
330 334	+0 175	−0 285	−0 189	−0 432*	−0 325	−0 270
400 416	+0 180	−0 038	+0 020	−0 033	+0 194	+0 018
420	+0 219	+0 103	−0 157	−0 186	−0 186	−0 323
421	0 319*	+0 009	−0 014	−0 187	+0 161	−0 015
42?	0 591**	−0 109	−0 259	−0 372*	−0 243	−0 299
Total mortality	0 235	−0 051	+0 058	−0 204	+0 370	−0 314

the middle age group and to the concentration of HCO_3 in the oldest

These rather startling findings forced us to analyze the group other degenerative heart diseases (422) in greater detail

Table IX shows the actual number and the number as a percentage of

TABLE VI Correlation coefficients (r) for death rate from all causes against all water parameters.

Code No	MnO_4^- consumption	Total solids	Ignition residue	Water hardness	Ca^{2+}	Mg^{2+}
1—8	−0.035	−0.085	−0.153	−0.244	−0.279	−0.224
140—150	−0.159	+0.003	+0.007	+0.021	+0.171	−0.197
151	+0.096	+0.004	−0.076	−0.111	+0.063	+0.282
152—161	−0.023	−0.182	−0.057	+0.019	−0.130	−0.383
162—163	−0.008	+0.057	−0.007	+0.048	+0.250	−0.319
164—165	−0.102	−0.048	−0.051	+0.013	+0.021	−0.163
170	−0.328*	+0.405*	+0.495***	+0.481***	+0.282	+0.317
171—205	+0.106	+0.118	+0.175	+0.261	+0.142	+0.299
330—334	+0.225	−0.354*	−0.377*	−0.432**	−0.385*	−0.219
400—416	+0.152	−0.018	−0.076	−0.033	+0.186	−0.321
420	−0.078	−0.212	−0.199	−0.186	−0.025	−0.031
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430—447	+0.336*	−0.250	−0.180	−0.146	−0.340*	−0.229
450—468	+0.195	+0.084	+0.044	+0.138	+0.284	+0.190
470—527	+0.091	+0.037	+0.093	+0.095	−0.025	−0.022
810—962	−0.128	−0.114	−0.140	−0.161	−0.141	−0.173
963—999	+0.103	+0.173	+0.232	+0.261	+0.108	+0.131
Other causes	+0.318*	−0.157	−0.059	−0.087	−0.252	+0.008
Total mortality	+0.326*	−0.256	−0.194	−0.204	−0.279	−0.032

females a corresponding correlation increasing with age was noticed in age group 45—64 years $p < 0.01$, and in the group 65—74 years $p < 0.005$ For none of the sex- and age groups was any correlation found between death rate from arteriosclerotic heart disease (420) and concentration of Ca^{2+}

The same relation is illustrated in figs 2 and 3 The death rates from arteriosclerotic heart disease (420) and 'other degenerative heart diseases' (422) for the age group 25—74 years, both sexes together, were plotted against total water hardness and concentration of Ca^{2+}, respectively The slopes of the calculated regression lines are highly significant ($p < 0.005$) for 422 against both water parameters and significant ($p < 0.01$) for 420 against water hardness, but not against concentration of Ca^{2+}

The multiple regression analysis showed no significant correlation between any of the water parameters (MnO_4^- consumption, concentrations of Ca^{2+}, HCO_3^-, SO_4^{2-}, Cl^-, and pH) and death rate from cause 420, with the exception of concentration HCO_3^- in the youngest males The death rate from cause 422 was found to be significantly correlated to concentration of Ca^{2+} in all the male groups, and in the youngest group also to Cl^- and pH For the females the death from cause 422 was significantly correlated to pH and concentration of Ca^{2+} in

TABLE VIII Correlation coefficients (r) for death rate from different groups of cardiovascular diseases and total mortality against concentration of Ca[1]

Code No	25—44 years		45—64 years		65—74 years	
	Males	Females	Males	Females	Males	Females
330—334	+0.100	−0.193	−0.258	−0.385*	−0.169	−0.300
400—416	+0.239	+0.011	+0.198	+0.186	+0.248	−0.006
420	−0.201	+0.035	+0.004	−0.025	−0.016	−0.056
421	+0.398*	+0.091	+0.092	−0.006	+0.216	+0.167
422	−0.490 *	−0.006	−0.486 **	−0.396	−0.458**	−0.516 **
Total mortality	−0.041	−0.071	+0.021	−0.279	−0.301	−0.367*

TABLE IX. Death rates in arteriosclerotic heart disease (420) and other degenerative heart diseases (422) in total material

Cause of death	25—44 years		45—64 years		65—74 years	
	Males	Females	Males	Females	Males	Females
420 No of deaths	322	49	6 697	1 897	7 180	5 004
% of total mortality	4.4	1.0	22	8.6	23	18
422 No of deaths	113	57	1 697	943	3 212	2 674
% of total mortality	1.5	1.1	5.5	4.3	10	9.4
Ratio 420/422	2.9	0.9	4.0	2.0	2.2	1.9
Total No of deaths	7 325	5 106	30 844	22 008	31 461	28 505

plot of the death rate from causes 420 and 422 in Stockholm shows (fig 5) that the change in the assignment of the diagnosis numbers refers mainly to the males

In view of this tendency it seemed worth while to analyze whether the correlation of mortality from No 422 to low calcium concentration was more marked in the beginning or at the end of the period studied The results are given in table X This table substantiates the over all negative correlation but

TABLE X Correlation coefficients (r) for five year mean values of death rate from other degenerative heart diseases (422) against concentration of Ca[1]

Group	1951—55	1956—60
Males 45—64 years	−0.218	−0.478
Males, 65—74 years	−0.429	−0.268
Females, 45—64 years	−0.566	−0.160
Females 65—74 years	−0.456	−0.363

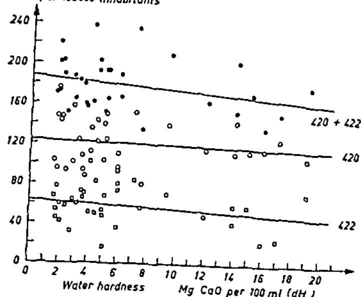


Fig 2 The mean death rates from arteriosclerotic heart disease (420) and other degenerative heart diseases' (422) for the age group 25—74 years, males and females together plotted against mean total water hardness

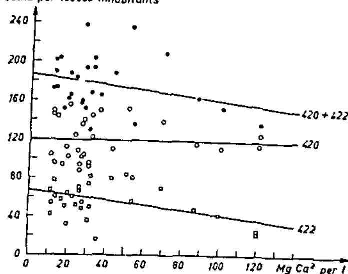


Fig 3 The mean death rates from arteriosclerotic heart disease (420) and other degenerative heart diseases (422) for the age group 25—74 years males and females together plotted against mean concentration of Ca^{++}

total mortality, of patients in the various age groups and sexes whose causes of death were registered as arteriosclerotic heart disease and other degenerative heart diseases", respectively On an average the ratio of the diagnoses 420 422 in males is 3 1, whereas in females it is closer to 3 2

There is, however, a strong tendency in later years towards an increased assignment of 420 in preference to 422 This fact is indicated in figs 4 and 5 The ratio for the number of deaths from 420/422 in the three biggest cities shows a three to fourfold increase during the 10 year period, as seen in fig 4 The

were thrown Patients not suffering from clear cut angina pectoris or myocardial infarction, but eventually dying unexpectedly or with heart failure in the absence of valvular heart disease or hypertension, have been referred to this group Recent puritanism in the diagnostic nomenclature has caused a gradual giving up of that diagnosis in preference to 420 — arteriosclerotic heart disease And now it turns out that — whatever the causal relationship involved may be — this group 422 repeatedly shows homogeneity in this statistical analysis of fairly large numbers This is true of our study but it is also true of the British study (7)

In both studies, the negative correlations with water hardness or calcium ion seem to be stronger for 422 than for 420 If we believe 420 to be a fairly homogeneous group 422 would also have to be accepted in the same way And if so — what then is the medical significance, the aetiology and the pathogenesis of other degenerative heart diseases ?

Summary

A study has been made on the relationship between deaths from cardiovascular diseases and various parameters in drinking water in 34 Swedish towns during 1951—60

The figures indicate a highly significant negative correlation between the calcium ion concentration and the statistical group 422 ("other degenerative heart diseases)

Eventually the findings confirm results of similar studies from other countries It does also raise the problem of the medical significance of the group 422

Acknowledgements

We are indebted to the Swedish Association against Heart and Lung Diseases for financial support and to Statistiska Centralbyrån *for considerable aid in the statistical analysis of the* material.

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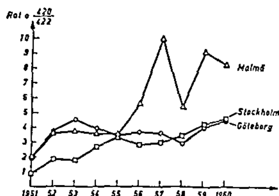


Fig 4 The ratio for the number of deaths from arteriosclerotic heart disease (420) and other degenerative heart diseases' (422) in the three biggest cities plotted for each year

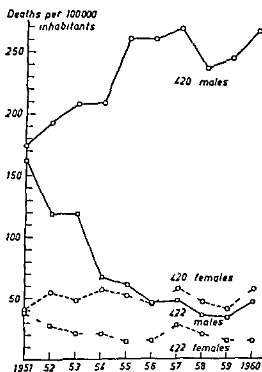


Fig 5 The death rate from arteriosclerotic heart disease (420) and other degenerative heart diseases (422) for the age group 45—64 years males and females separately, in Stockholm plotted for each year

unfortunately does not give a definite answer to the said question Further studies, therefore, are necessary on this point

Discussion

The results presented in a general sense agree with those published from Japan, U S and England (5, 7, 12, 13, 14) Once more, in still another part of the world, in a country with a very homogeneous and stationary population, which is reasonably well organized as far as population statistics and water supply are concerned, the very strange fact has been revealed that 'water hardness" and some constituents of drinking water associated with water hardness are in one way or another correlated to a specific fraction of the death rate in cardiovascular diseases

The interpretation of this fact has been discussed in some detail by earlier investigators, and various suggestions, e g concerning the possible role of certain trace elements deriving from soil or water pipes etc , have been made

We have great difficulty in explaining why the calcium concentration in water should affect cardiovascular mortality, when the relationship between calcium content of water and of food is considered It appears to us most reasonable to regard calcium as an indicator" of something else, which might be a causal agent Trace metals may be one type of factor that could be operating Other environmental factors might also play a role These problems will require further study

Another aspect which has been brought out by this study is the question of the homogeneity of the statistical group 422 — other degenerative heart diseases" This group has long been regarded as a waste paper basket into which doubtful and difficult diagnostic problems

were thrown Patients not suffering from clear cut angina pectoris or myocardial infarction, but eventually dying un expectedly or with heart failure in the absence of valvular heart disease or hypertension, have been referred to this group Recent puritanism in the diagnos tic nomenclature has caused a gradual giving up of that diagnosis in preference to 420 — arteriosclerotic heart disease And now it turns out that — whatever the causal relationship involved may be — this group 422 repeatedly shows homogeneity in this statistical analysis of fairly large numbers This is true of our study but it is also true of the British study (7)

In both studies the negative correla tions with water hardness or calcium ion seem to be stronger for 422 than for 420 If we believe 420 to be a fairly homo-geneous group 422 would also have to be accepted in the same way And if so — what then is the medical significance the aetiology and the pathogenesis of other degenerative heart diseases ?

Summary

A study has been made on the relation ship between deaths from cardiovascular diseases and various parameters in drinking water in 34 Swedish towns during 1951—60

The figures indicate a highly signifi cant negative correlation between the calcium ion concentration and the statistical group 422 (other degenera tive heart diseases)

Essentially the findings confirm results of similar studies from other countries It does also raise the problem of the medical significance of the group 422

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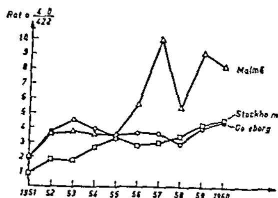


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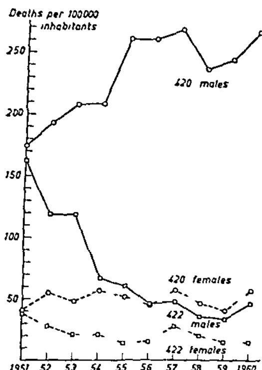


Fig 5 The death rate from arteriosclerotic heart disease (420) and other degenerative heart diseases (422) for the age group 45—64 years males and females separately in Stockholm plotted for each year

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Acta Medica Scandinavica Vol 178, fasc 2, 1965

From the First Medical Service, Sahlgrenska sjukhuset, University of Goteborg Goteborg, Sweden

The Effect of Lactic Acid on Adipose Tissue Metabolism in Vitro

By

PER BJÖRNTORP

Norepinephrine and other catecholamines cause an increase of plasma free fatty acids (FFA) (8), by an increased lipid mobilization from adipose tissue depots This corresponds *in vitro* to an increased outflow of both FFA and glycerol from surviving adipose tissue (6) The trigger mechanism for these lipolytic agents seems to be a lipase possible to activate *in vitro* (9 3 10 1)

When screening different agents as far as their effects on the lipase activation by norepinephrine it was found that lactic acid caused an inhibition of this activation Because of the possible importance of this finding for the present concept of regulation of lipid mobilization, it was considered advisable to report these findings separately

Materials and methods

Male 200 300 g rats of a Sprague Dawley strain Anticimex were killed by a blow on the head and decapitation The epididymal fat pads were immediately removed placed in 0 15 M KCl at room temperature and the distal end dissected into two approximately equal halves. These were then incubated in 4 % albumin (Armour Fraction V) and 10 mM glucose in Krebs-Ringer bicarbonate solution pH 7 4 at 37 C during 90 minutes Glycerol (4) and FFA (5 2) of the incubation medium was determined at 0 and 90 minutes. Lactate was added from beginning of incubation to a final concentration of 30 or 3 mM in the form of the lithium salt (British Drug House) Norepinephrine (0 1 μg/ml) was added 10 minutes before ending incubation

The tissues were then removed from the incubation media rinsed in 0 15 M KCl homogenized and assayed for lipase activity as described by Vaughan et al (10)

Submitted for publication March 10 1965

Results

Tables I and II give the results for lactate in the concentration of 3 and respectively 30 mM Norepinephrine increased glycerol and FFA concentrations in the incubation medium and also lipase activity ($< 0 01$) Lactate at 3 mM concentration inhibited the norepinephrine stimulated outflow of glycerol and FFA to the medium as well as lipase activity (table I $p < 0 05$) and at 30

12 SCHROEDER, H A Degenerative cardiovascular disease in orient hypertension J Chron Dis *8* 312, 1958
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The possibility of lactate inhibition of adipose tissue lipolysis seems to be of interest in conditions, where pulmonary or circulatory insufficiency causes accumulation of lactic acid in plasma. Of particular interest in this regard are questions related to obesity and physical training.

Summary

Lactic acid in concentrations occuring in plasma *in vivo* inhibited norepinephrine increase of fatty acid and glycerol outflow as well as lipase activity in rat epididymal fat pads *in vitro*.

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TABLE I The effect of 3 mM lactate on norepinephrine stimulated glycerol and free fatty acid outflow and lipase activity in rat epididymal fat pads in vitro Means ± S D for 7 experiments

	Glycerol (μEq/g/90)	FFA (μEq/g/90)	Lipase activity (μEq FFA/g/h) O lactate in assay	Lactate in assay
O	0.44 ± 0.12	0.6 ± 0.2	12.6 ± 3.0	12.0 ± 3.1
Norepinephrine	0.90 ± 0.13	2.4 ± 0.7	21.4 ± 3.3	22.1 ± 4.9
Norepinephrine + Li lactate	0.71 ± 0.12	1.7 ± 0.3	17.4 ± 2.6	18.2 ± 3.7
Norepinephrine + Li Cl	0.94 ± 0.10	2.3 ± 0.6	21.0 ± 5.1	24.2 ± 5.1

TABLE II The effect of 30 mM lactate on norepinephrine stimulated glycerol outflow and lipase activity in rat epididymal fat pads in vitro Means ± S D of 9 experiments

	Glycerol (μM/g/90)	Lipase activity (μEq FFA/g/h)
O	0.48 ± 0.11	11.1 ± 3.0
Norepinephrine	0.95 ± 0.20	24.9 ± 5.1
Norepinephrine + Li lactate	0.72 ± 0.14	18.0 ± 4.5
Norepinephrine + Li Cl	1.00 ± 0.12	25.2 ± 4.5

mM concentration glycerol outflow and lipase activity (table II, $p < 0.02$) Lithium chloride had no effect on the norepinephrine stimulated activity, showing that lactate was responsible for the inhibition Lactate in the assay system had no effect as seen in table I

Discussion

It was thus shown, that lactate inhibits the norepinephrine increase of FFA and glycerol outflow from adipose tissue and also the lipase activity, sensitive for hormone stimulation There was no effect on preformed lipase activity, demonstrating, that its effect is probably exerted on the lipase activation mechanism In this regard it has a similar effect to nicotinic acid as described recently (2)

Issekutz has shown, that infusion of lactate to the dog decreases plasma FFA and increases specific activity of plasma FFA during constant infusion of labelled FFA (7) This indicates a decrease of outflow of FFA from fat depots Such a decrease caused by lactic acid has also been suggested to occur during work (7)

The lactate concentrations shown to inhibit adipose tissue lipolysis are within the physiological concentration range in plasma, occuring for example during work It therefore seems quite possible, that the findings *in vitro* should correspond *in vivo* Theoretically it seems suitable with a messenger such as lactic acid, indicating a relative oxygen deficiency in working muscles and inhibiting adipose tissue outflow of fatty acids, which are broken down exclusively aerobically

Acta Medica Scandinavica. Vol 178, fasc 3, 1965

From the Departments of Medicine (Head G Biorck M D) and Clinical Physiology (Head B Pernow M D), Karolinska Institutet at Serafimerlasarettet Stockholm, Sweden

Potassium Infusion in Coronary Disease

By

E Orinius

Gubner (1) found that an infusion of potassium chloride prior to performance of exercise reduced the ST depressions in [illegible]

this theory has been increasingly questioned in recent years In the light of the theory of hyperkalemic venous spasm as [illegible] the possibility by reducing a [illegible] coronary veins [illegible]hould nitro-glyc- [illegible]ong an arm vein, [illegible]rt to this hypoth[illegible] [illegible]is possibility was [illegible]his investigation

CORRIGENDUM

Acta Medica Scandinavica. Vol. 178, fasc. 3, 1965

Author	Paper	Page	Text
Carl J [illegible]han [illegible] the	Pine pollen pneumoconiosis in the rat and its relation to sarcoidosis	377	Table I should read Lung weight (% of animal body weight)

[illegible]ls

[illegible]atients 5 male and [illegible]ho had shown post exercise ST T-changes of the type of suspect or definite coronary insufficiency (3) and because of that had been further examined by coronary angiography The angiogram had in the 5 males demonstrated total occlusion in one two or all the three of the main coronary arteries while the 3 females had normal coronary angiograms (table I) None of the patients had evidence of congenital or rheumatic heart disease arterial hypertension or preexcitation syndrome nor were any receiving cardiac glycosides.

[illegible] spasm which had been provoked by a local hyperkalemia because of an abnormal potassium leakage from hypoxic cells As a support for this theory it is stated among other things that a rapid potassium infusion in an antecubital vein causes spasm and pain along the subclavian vein

The effect of nitroglycerin on the coronary ischemic pain has for a long time been thought to depend on dilatation of the coronary arterial system but

In each patient the exercise test study started with a bicycle ergometer examination

Submitted for publication February 22 1965

Book reviews

The transport of lymph in man By R Blomstrand, C Franksson and B Werner 80 p 27 ill Stockholm 1965

In this little condensed monograph the authors — Blomstrand of the department of clinical chemistry, Franksson and Werner of the department of surgery at Serafimerlasarettet, Stockholm — have presented their findings from recent experimental and clinical studies of lymph from the thoracic duct

After a few introductory and historical notes, the authors describe the cannulation of the thoracic duct in man, which they have used in about hundred patients In the following chapters, the composition of lymph, the lymphatic transport of lipids and fat solute vitamins and the diagnostic and therapeutic uses of lymph drainage are discussed on the basis of vast personal experience. Of salient interest to the clinician are the findings of cancer cells in thoracic duct lymph in malignant disease, and the symptomatic treatment of chronic lymphatic leukemia by lymph fistula, as well as removal of lymph in cases of kidney transplantation in order to diminish or abolish graft rejection, which is mediated at least partly by lymphocytes The method may also offer possibilities in patients with liver cirrhosis and portal hypertension

Although the authors rightly stress, that many of their observations are preliminary and in need of extension, the presentation is unique and provocative and well worth reading

Gunnar Biörck
Stockholm

The coronary arteries Arteriography micro-anatomy, and pathogenesis of obliterative coronary artery disease By William F M Fulton 354 p Charles C Thomas Publ, Springfield, Ill 1965

A detailed monography of atherosclerosis in the coronary arteries, especially its pathogenesis Outstanding illustrations including arteriography (predominantly stereoscopic photographs), microanatomy and histopathology

The author has an extremely elegant style and compelling powers of exposition He advances plentiful morphological evidence for the view that many atherosclerotic lesions arise from thrombotic formations in the arterial lumen

This imposing work is warmly recommended to pathologists cardiologists and others

Åke G H Lindgren
Stockholm

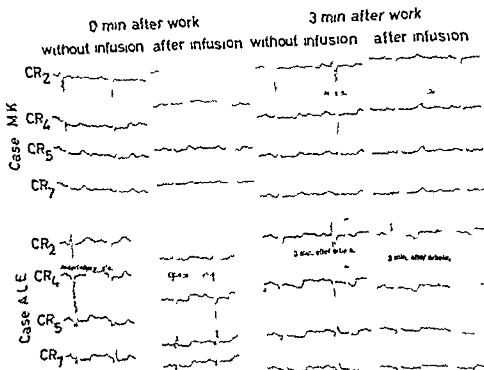


Fig. 1. Post-exercise ECGs in 2 patients in the exercise study

Nitroglycerin study

The nitroglycerin study was conducted in 8 additional patients, 5 male and 3 female with recent myocardial infarction or classical symptoms of angina pectoris (table II).

An identical potassium-containing solution was infused in a distal forearm vein with the patient in the supine position. The infusion rate was regulated so that the pain produced extended from the infusion point to the upper arm. After the pain had been at a constant level for approximately 5 minutes a placebo tablet was administered to the patient sublingually. Five minutes later the patient was asked to point out the upper pain limit again and then a 0.5 mg nitroglycerin tablet was given sublingually. Five minutes later the upper pain limit was again indicated. At the same time the patient was asked about headache and facial burning as a sign of nitroglycerin action. The infusion rate was kept constant during the whole time and varied among the patients from 64 to 168 drops/min.

Results

Exercise study

During the exercise test without a preceeding potassium glucose infusion all the patients had ST-depressions of at least 0.7 mm in one or more of the leads CR_{4-7} during the work and these changes remained greater than 0.5 mm in all cases in the electrocardiogram immediately after work and in all cases except one 3 minutes after exercise. The ST-depressions after exercise were of the horizontal or downward sloping type (fig. 1

TABLE I The exercise material

Patient	Sex	Age	Angina pectoris	Myocardial infarctions (no)	Exercise test tolerance (kpm/min) (min)	ECG 3 after exercise: S T J depression (mm)	ECG 3 after exercise: Sloping	ECG 3 after exercise: Lead	Coronary angiogram (no main branches severely obstructed)
A C	♂	55	Typical	0	450 (2)	1 5	Downward	CR_4	3
P I	♂	48	Typical	1	150 (3)	1 5	Downward	CR_4	2
S W	♂	55	Typical	0	300 (6)	0 7	Upward	CR_4	3
F C	♂	51	Typical	0	600 (6)	0 5	Downward	CR_7	1
E R	♂	47	0	1 ?	600 (6)	1 5	Upward	CR_4	3
M K	♀	39	Atypical	0	600 (3)	1 0	Horizontal	CR_4	Normal
A W	♀	50	Atypical	0	400 (2)	1 0	Horizontal	CR_4	Normal
A L E	♀	29	Atypical	0	200 (6)	0 7	Downward	CR_4	Normal

TABLE II The nitroglycerin study

Patient	Sex	Age	Diagnosis	Infusion rate (drops/min)	Distance needle-proximal pain limit (cm): Before tablets	Distance needle-proximal pain limit (cm): After blind tablet	Distance needle-proximal pain limit (cm): After 0 5 mg nitroglycerin	Headache/burning after nitroglycerin
A A	♂	68	Post myocardial infarction	160	12	20	0	+
E B	♂	50	Post myocardial infarction	90	20	20	0	+
K S	♂	65	Post myocardial infarction	72	32	21	16	+
M B	♀	72	Post myocardial infarction	130	26	26	8	+
E T	♂	46	Effort angina	150	33	33	20	+
S B	♂	47	Post myocardial infarction	168	29	24	3	+
K S	♀	69	Post myocardial infarction	64	23	15	16	−
F P	♀	68	Post myocardial infarction	80	19	19	14	+

according to Sjostrand (4) This was carried on to a load, where the ECG showed ST depressions of at least 0 7 mm or the patients developed angina pectoris pain One or two days later the test was repeated at the same hour and with the same load after an intravenous infusion of 30 mEq potassium chloride in 500 ml 5 5 % glucose solution The infusion was completed under electrocardiographic monitoring at as rapid a rate as could be tolerated with regard to the pain produced in the infusion arm The infusion times thus varied between 33 and 70 minutes The venous serum potassium concentration in the non infusion arm was determined before and after the infusion

		0 after exercise			3 after exercise	
Infusion	Lead[1]	Control	Infusion	Lead[1]	Control	Infusion
2 0	CR_4	1 8	2 0	CR_4	1 2	1 3
0 3	CR_6	0 5	0 7	CR_5	0 8	0 8
2 5	CR	1 2	1 2	CR_5	0 2	0 2
3 0	CR_4	2 7	2 3	CR_4	2 0	2 5
2 5	CR_5	1 5	1 7	CR_6	1 5	1 [illegible]
m 2 [illegible]		m=1 5	m=1 6		m=1 1	m=1 3
2 0	CR_5	1 5	1 5	CR_5	1 0	0 7
1 5	CR_4	1 5	(0 8)	CR_4	1 0	(0)
	CR_4	(1 2)	1 5	CR_5	(0 5)	0 [illegible]
1 5	CR_4	1 2	1 [illegible]	CR	0 8	0 7
m [illegible]		m=1 4	m=1 3		m=0 8	m=0 5

Discussion

Exercise study

Gubner's finding that pretreatment with an infusion of 30 mEq potassium chloride in 500 ml 5 5 % glucose solution reduces the ST depressions of a post exercise electrocardiogram has not been reproduced The only known difference between Gubner's conditions and those of this study concerns the infusion time Here it varied between 33 and 70 minutes An infusion rate as fast as could be tolerated by the patient was used However due to the arm pain produced at rapid rates in only 3 cases was it possible to decrease the total time of administration to between the 20 minutes that Gubner mentions in one paper 2 and the 40 minutes in another (1) It is impossible to determine whether this difference in infusion time can be responsible for the difference in results here reported and those of Gubner as his reports contain information neither about the frequency of influenceable electrocardiograms nor about variations in infusion time

Nitroglycerin study

The nitroglycerin study indicates that this substance can relieve the hyperkalemia induced pain of a peripheral vein Whether this occurs by relieving a spasm of the vein and or by changing the central or peripheral circulation in such a way that the infused potassium becomes more diluted has not been in-

TABLE III The exercise results

Patient	Work load (kpm/min)	Infusion time (min)	Serumpotassium (mEq/l)		S T J-depressions (mm)	
					During exercise	
			Before infusion	After infusion	Lead[1]	Control
Pathological coronary angiogram						
A C	200 (6)	55	—	—	CR₄	2 7
P I	150 (6)	38	4 7	5 0	CR₄	0 7
S W	300 (6)	70	4 5	5 0	CR₄	2 0
F C	600 (6)	35	3 9	4 4	CR₄	2 7
E R	400 (6)	51	4 5	4 6	CR₄	2 3
						m=2 1
Normal coronary angiogram						
M K	400 (6)	50	—	—	CR₄	2 0
A W	200 (6)	55	—	—	CR₄	2 0
A L E	600 (6)	33	3 9	4 3	CR₄	1 8
						m=1 9

[1] Lead with maximal depression

of the 5 cases with pathological coronary angiograms and in 2 of the cases with normal appearing angiograms After pretreatment with potassium-glucose-infusion there was no consistant change of the post exercise ST depressions The extremes were a reduction of 0 5 mm in one case and an increase of 0 5 mm in another (table III and fig 1) The mean of the serum potassium concentration in 5 examined cases was 4 3 mEq/l before the infusion and 4 7 mEq/l after it (table III)

Nitroglycerin study

During the potassium infusions it was noted that the pain began to extend along the arm from the infusion point or just above after 1 or 2 minutes By varying the infusion rate the upper pain limit could be moved from the forearm, upper arm even into the axilla Usually there were minor changes of the upper pain limit after the placebo tablet

After nitroglycerin all the patients except one reported headache or facial burning Six of the 8 patients indicated a greater retraction of the upper limit after nitroglycerin than after placebo tablet There was no significant difference between the mean level before tablets and that after a placebo tablet ($p \geqslant 0\ 1$) but a significantly lower mean level after nitroglycerin compared with that after placebo tablet ($p < 0\ 01$) One of the non reacting patients had no head sensations after nitroglycerin

Acta Medica Scandinavica. Vol 178, fasc 3, 1965

From the Hematology Section Medical Department A Rigshospitalet, University of Copenhagen Copenhagen, Denmark

Proliferative Activity of Blast Cells in Leukemia and Myelofibrosis

Morphological Differences Between Proliferating and Non proliferating Blast Cells

By

Sven Aage Killmann

The single most striking feature of leukemia is the accumulation of immature white cells in blood and hemopoietic tissues It is a prerequisite for the diagnosis of leukemia that at some stage of the disease abnormal numbers or types of white cells can be demonstrated Clinical symptoms however appear to be much more closely related to the lack of normal functioning cells in the blood than to the presence of immature white cells In fact the accumulation of leukemic cells may merely be an epiphenomenon of leukemia Nevertheless it is generally held that in some ill-defined manner the presence of leukemic cells is causally related to the inadequacy of normal hemopoietic cell production Perhaps the best support for this belief is the general experience that a full remission of leukemia restoration of normal hemopoietic conditions is not obtained unless the leukemic cells are so much reduced in number as to appear "eradicated Therefore, current chemotherapy of leukemia is primarily aimed at inhibiting the proliferation of leukemic cells During recent years, the kinetics of leukemic cells has been studied with various technics Through insight into the differences between the proliferation of normal and leukemic white cells a better understanding of the pathophysiology of leukemia may be obtained which perhaps will help to a more judicial use of therapy

In a previous study utilizing H^3-thymidine labeling *in vivo* some information about the life cycle of leukemic cells was obtained (24) The evaluation of such *in vivo* studies is so time consuming as to preclude studies of larger groups of patients The primary purpose of the present study was to try to establish the general validity of some of the previously obtained observations by examining the uptake of H^3 thymidine in blood and bone marrow in a group of patients with leukemia and myelofibrosis In addition

Submitted for publication February 18, 19[illegible]

vestigated Coronary venule walls are also known to contain muscular elements and thus either or both of these mechanisms might be active there That this might partially explain the pain relieving ability of nitroglycerin in angina pectoris merits further consideration

Summary

Eight patients with angina pectoris (5 with pathological and 3 with normal coronary angiogram) underwent bicycle exercise test with and without pretreatment with an intravenous infusion of 30 mEq potassium chloride in 5 5 % glucose This pretreatment had no appreciable effect on the ST-depressions

In 8 patients with angina pectoris or recent myocardial infarction the pain in the infusion arm during a potassium-glucose treatment significantly retracted after 0 5 mg nitroglycerin sublingually compared with a placebo One explanation of this finding could be that nitroglycerin acts on the (hyperkalemic) vein, and this raises the question of whether a similar mechanism cannot also function in angina pectoris.

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Proliferative Activity of Blast Cells in Leukemia and Myelofibrosis

Morphological Differences Between Proliferating and Non proliferating Blast Cells

By

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The single most striking feature of leukemia is the accumulation of immature white cells in blood and hemopoietic tissues It is a prerequisite for the diagnosis of leukemia that at some stage of the disease abnormal numbers or types of white cells can be demonstrated Clinical symptoms however appear to be much more closely related to the lack of normal functioning cells in the blood than to the presence of immature white cells In fact the accumulation of "leukemic cells may merely be an epiphenomenon of leukemia Nevertheless it is generally held that in some ill-defined manner the presence of leukemic cells is causally related to the inadequacy of normal hemopoietic cell production Perhaps the best support for this belief is the general experience that a full remission of leukemia (restoration of normal hemopoietic conditions) is not obtained unless the leukemic cells are so much reduced in number as to appear "eradicated Therefore, current chemotherapy of leukemia is primarily aimed at inhibiting the proliferation of leukemic cells During recent years, the kinetics of leukemic cells has been studied with various technics Through insight into the differences between the proliferation of normal and leukemic white cells a better understanding of the pathophysiology of leukemia may be obtained which perhaps will help to a more judicial use of therapy

In a previous study utilizing H^3-thymidine labeling *in vivo* some information about the life cycle of leukemic cells was obtained (24) The evaluation of such *in vivo* studies is so time consuming as to preclude studies of larger groups of patients The primary purpose of the present study was to try to establish the general validity of some of the previously obtained observations by examining the uptake of H^3 thymidine in blood and bone marrow in a group of patients with leukemia and myelofibrosis In addition

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vestigated. Coronary venule walls are also known to contain muscular elements and thus either or both of these mechanisms might be active there. That this might partially explain the pain relieving ability of nitroglycerin in angina pectoris merits further consideration.

Summary

Eight patients with angina pectoris (5 with pathological and 3 with normal coronary angiogram) underwent bicycle exercise test with and without pretreatment with an intravenous infusion of 30 mEq potassium chloride in 5.5 % glucose. This pretreatment had no appreciable effect on the ST-depressions.

In 8 patients with angina pectoris or recent myocardial infarction the pain in the infusion arm during a potassium-glucose treatment significantly retracted after 0.5 mg nitroglycerin sublingually compared with a placebo. One explanation of this finding could be that nitroglycerin acts on the (hyperkalemic) vein, and this raises the question of whether a similar mechanism cannot also function in angina pectoris.

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TABLE I Acute leukemia percentage of leukemic blast cells labeled with H^3 thymidine in vitro Cases 8—10 had medication for 1—4 days, cases 11 15 and 20 for 11—21 days

Case	Sex and age		Type	Blasts labeled (%) Blood	Marrow	Blast count (per μl)	Disease duration (months) (before/after study)	Treatment before study	Enlarged Liver	Spleen
1	♂	68	AML	6 34	17 25	34 000	2/8	Nil	+	—
2	♂	66	—	5 0	—	220 000	?/0 03	Nil	—	+
3	♀	58	—	4 5	8 25	134 000	1/0 5	Nil	+	—
4	♂	39	—	2 0	9 0	800	5/0 75	Nil	—	+
5	♂	53	—	1 76	10 3	33 000	1/2	Nil	+	—
6	♂	68	—	1 33	—	900	18/1	Nil	++	—
7	♂	63	—	0 75	11 3	1 000	1/1	Nil	—	—
8	♂	59	—	8 0	—	160 000	0 5/0 06	6 MP P	++	—
9	♀	54	—	7 3	—	7 200	6/7	6 MP P	+	++
10	♀	23		2 53	7 6	135 000	0 5/0 13	P	++	—
11	♀	76	—	0 4	9 2	4 200	3/0 5	P	—	—
12	♂	47	AMoL	0 6	4 1	32 000	1 25/>10	Nil	—	—
13	♀	18	AEL	2 0	—	3 200	7/3	Nil		—
14	♂	56	SAML	6 26		2 200	2 5/6	Nil	—	—
15	♀	49	—	8 7	—	30 000	1/5	6 MP P	++	—
16	♀	62	ALL	4 0	15 3	300	5/2	Nil	—	—
17	♀	15		3 5	—	4 500	1 25/9	Nil	+	++
18	♀	51	—	1 79	—	1 000	5/1	Nil	—	—
19	♂	46	—	0 67	8 3	200	1/5	Nil	—	—
20	♀	13	—	3 25	—	5 600	?/0 75	P	—	—

AML acute myeloblastic leukemia AMoL = acute monocytic leukemia AEL = acute erythroleukemia (di Guglielmo) SAML — subacute myeloid leukemia ALL = acute lymphoblastic leukemia 6MP 6-mercaptopurine P = prednisone Organ enlargement — = not palpable maximally 3 fingersbreadth below costal margin ++ = approximately to level of umbilicus

radiograms the cytoplasmic granulation of the more mature neutrophil precursors does not show and differentiation must be based on the structure and shape of the nucleus and the color of the cytoplasm In acute leukemia and in myelofibrosis differentiation has not been a problem In advanced chronic myeloid leukemia the distinction is more difficult because granulated neutrophilic precursors typically have a less coarse chromatin than in normal granulocytopoiesis In counts of autoradiograms of chronic myeloid leukemia only cells with a strictly leptochromatic nucleus have been classified as myeloblasts

Statistics for correlation studies Spearman s rank-order correlation was used (28)

Results

1 Labeling Indices

The results are detailed in tables I and II which also include other pertinent data

evidence will be presented that subtle morphological differences exist in blast cells which appear related to their capacity for further division Finally, some aspects of leukemic blast cell proliferation will be considered

Material

Thirty three patients with circulating blast cells have been studied The series includes myelofibrosis (5 cases), chronic myeloid leukemia (8 cases), and acute leukemia (20 cases) In acute leukemia, it was attempted to differentiate morphologically between myeloblastic and lymphoblastic leukemia Blast cells were classified as myeloblasts when the nuclear chromatin was finely granular and when wide variations with respect to cell size, nucleocytoplasmic ratio and nuclear shape were present Cells with slightly clumped chromatin, round and uniform nuclei, scanty cytoplasm, and little variation in nucleocytoplasmic ratio were diagnosed as lymphoblasts One patient had typical acute erythroleukemia (di Guglielmo), one had acute monocytic leukemia (Schilling type), and in two patients large numbers of atypical promyelocytes and myelocytes were present in blood and marrow besides the myeloblasts (subacute myeloid leukemia)

Methods

Blood 3 5 ml of venous blood was withdrawn into a tube containing 0 5 ml of saline with heparin (heparin Leo® 1 mg/ml saline) 1 0 ml of 6 % dextran in 5 % glucose (Macrodex®), and 2—5 μC of H^3 thymidine (1 9 C/mM, Schwarz Bio Research Inc Orangeburg N Y) After mixing the tube was incubated at 37° C for 1 hour (in case of 2—2 5 μC H^3 thymidine added) or left at room temperature for 1 hour (in case of 5 μC H^3 thymidine added) Smears were then prepared from the buffy coat If the leukocyte count was low, the buffy coat was briefly centrifuged and the smears made from the cell sediment

It has been established that extension of incubation beyond 1 hour may increase the grain count of labeled cells but will not increase the percentage of cells which are labeled (22), nor will this percentage be influenced by the use of larger amounts of the tracer (44) With the amounts of tracer used, the labeling percentage is identical at room temperature and at 37° C (22)

Bone marrow was aspirated from the ilium into a syringe containing a few drops of saline with heparin (1 mg/ml) Approximately 1 ml of marrow was transferred to a tube containing 2 μC of H^3-thymidine After incubation at 37° C for 1 hour, smears of small bone marrow particles were prepared

Autoradiography the slides were dipped in Kodak NTB 2 emulsion diluted 1 1 with distilled water and exposed at 4° C Exposure time chosen was such as to produce the highest number of grains compatible with reliable cytological classification This was usually achieved by 7 days' exposure Occasionally, longer exposure was necessary After development (Kodak D 19 b developer for 2 minutes, Kodak X ray fixer for 2 minutes) the slides were stained with Giemsa at pH 5 75

Evaluation Background was less than 1—2 grains per nucleus Cells with 5 grains or more were considered labeled The vast majority of labeled cells had high grain counts, 25 grains or more Cells with grain counts just below or above the threshold adopted for classifying a cell as labeled occurred rarely, and varying the adopted (arbitrary) threshold within reasonable limits would therefore not significantly affect the labeling percentages found in this study

In buffy coat smears 300—1 500 blasts were counted except in a few instances where blasts were very scarce and only 100—200 were counted In bone marrow smears 300—1 000 blast cells were counted To avoid contamination with blasts from the blood areas were selected in which the marrow blasts clearly were part of a marrow particle

Since promyelocytes and myelocytes label more extensively than blast cells it is important that this distinction is made In auto-

TABLE III Blast cells in the blood differences in labeling index in morpholog cal subtypes. For descript on of types see text. No n parenthes s indicates no of cells in category counted Counts shown here do not enter into the fig ures for gross label ng ind ces of table I

	Label ng index of blast cell subtypes		
Case	Typical	Sl ghtly atyp cal	H ghly atyp cal
1	15 0 (300)	2 7 % (300)	0 % (200)
3	7 3 (300)	2 0% (400)	0% (200)

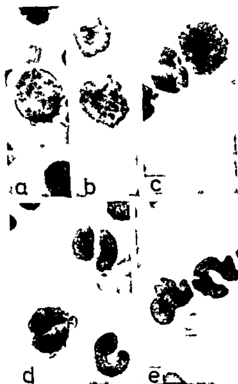


Fig 1 Illustrations of blast cell subtypes (autoradiographs) a Labeled typ cal blast b Labeled sl ghtly atyp cal blast. c Labeled typ cal blast and unlabeled h ghly atyp cal blast d. Labeled (gra ns out of focus) sl ghtly atyp cal blast (note gap traversing nucleus) two unlabeled h ghly atyp cal blasts the lower one has a metamyelocyte l ke appearance e Two h ghly atyp cal blasts

cases the blasts differ widely in nuclear outline and shape During the study it was noted that typical blasts appeared to be labeled more frequently than atypical blast cells with irregular nuclei The smears of acute leukemia were then re investigated with this in mind In most cases the vast majority of blast cells were typical with round and regular nuclei These cases were not studied further In 7 cases (nos 1 2 3 4 7 12 15) apprecia ble numbers of the blast cells in the blood (more than 40 % of all blast cells) were atypical with more or less irregular nu clear outline nuclear indentations or twisted nuclei Often these cells had a broader rim of cytoplasm than seen in the typical blast cells

In these cases the labeling index was determined separately in a) typ cal blast cells round nuclei with regular outline evenly distributed chromatin) b) slightly atyp cal blast cells (slightly irregular nuclear outline or small indentations chromatin evenly distributed or showing delicate lacy structure) and c) marked ly atyp cal blast cells (highly irregular nuclear outline deeply indentated or twisted nuclei sometimes lacy chromatin structure cells have a monocytoid appearance) Examples of the three blast cell categories are shown in fig 1 Obviously transitions between the groups exist and assignment of some cells to one group or the other will be arbitrary but nevertheless blast cell differential counts were fairly reproducible Representative

TABLE II Chronic myeloid leukemia and myelofibrosis percentage of blast cells in the blood labeled with H^3 thymidine in vitro

Case	Sex and age		Diagnosis	Blast count (per µl)	Blasts labeled (%)	Treatment	Enlarged	
							Liver	Spleen
21	♂	37	CML, early stage	10 000	17.5	Nil	++	+++
22	♀	69	—	2,200	19.0	Nil	—	—
23	[illegible]	42	CML, advanced	100 000	6.4	6 MP	+	+++
24	♂	25	—	43 000	10.3	6 MP P	—	+++
25	♂	25	—	13 000	12.25	6 MP P	—	+++
26	♂	59	—	100 000	16.25	6 MP, P	++	+++
27	♀	41	—	29 000	17.25	X-ray P	—	++
28	♂	38	—	4,000	20.0	6 MP, P	—	+++
29	[illegible]	70	Myelofibrosis	1 300	6.5	Nil	++	+
30	♀	65	—	1 700	5.3	Nil	++	+++
31	♂	77	—	750	2.5	Nil	+	++
32	♂	69	—	250	8	P	+	++
33	♂	45	—	500	4	P	++	—[1]

CML = chronic myeloid leukemia Organ enlargement +++ = organ extending beyond level of umbilicus Other signs and abbreviations as in table I

[1] Splenectomized

Blood

In acute lymphoblastic leukemia the labeling index (percentage of cells labeled with H^3-thymidine) of blast cells in the blood ranged from 0.67—4.0 %. In acute myeloblastic leukemia the index varied from 0.4—7.3 %. Blast cells in myelofibrosis (4—8 % labeled) were in the same range as acute myeloblastic leukemia, whereas myeloblasts in chronic myeloid leukemia labeled to a much larger extent (6.4—20 %, in 7 out of 8 cases above 10 %). In the two cases of subacute myeloid leukemia the labeling index (6.3—8.7 %) fell within the higher range of acute myeloblastic leukemia and the lower range of chronic myeloid leukemia.

Bone marrow

H^3-thymidine uptake was studied in 10 patients with acute leukemia. In myeloblastic leukemia, 8.25 — 17.25 % of myeloblasts were labeled. In two cases of lymphoblastic and in one case of monocytic leukemia, 15.3, 8.3 and 4.1 % of the blast cells were labeled, respectively. With no exception the labeling index was much higher in the marrow than in the blood.

2 LABELING OF TYPICAL AND ATYPICAL BLAST CELLS

Anybody familiar with the cytology of acute leukemia is aware of the fact that in some cases the blast cells are morphologically quite uniform whereas in other

TABLE III Blast cells in the blood differences in labeling index in *morphological* subtypes. For description of types see text No in parenthes s indicates no of cells in category counted Counts shown here do not enter into the fig ures for gross labeling indices of table I

	Labeling index of blast cell subtypes		
Case	Typical	Slightly atypical	Highly atypical
1	15 0 (300)	2 7% (300)	0 % (200)
3	7 3° (300)	2 0% (400)	0% (200)

cases the blasts differ widely in nuclear outline and shape During the study it was noted that typical blasts appeared to be labeled more frequently than atypical blast cells with irregular nuclei The smears of acute leukemia were then re investigated with this in mind In most cases the vast majority of blast cells were typical with round and regular nuclei These cases were not studied further In 7 cases (nos 1 2 3 4 7 12 15), apprecia ble numbers of the blast cells in the blood (more than 40 % of all blast cells) were atypical with more or less irregular nu clear outline nuclear indentations or twisted nuclei Often these cells had a broader rim of cytoplasm than seen in the typical blast cells

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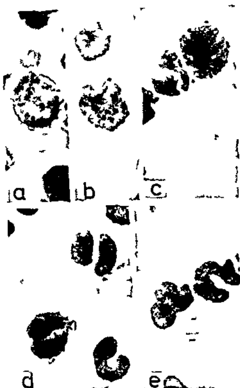


Fig 1 Illustrations of blast cell subtypes (autoradiographs) a Labeled typical blast b Labeled slightly atypical blast. c. Labeled typical blast and unlabeled highly atypical blast d Labeled (grains out of focus) slightly atypical blast (note gap traversing nucleus) two unlabeled highly atypical blasts the lower one has a metamyelocyte like appearance e Two highly atypical blasts

ly atypical blast cells (highly irregular nuclear outline deeply indentated or twisted nuclei sometimes lacy chromatin structure cells have a "monocytoid appearance) Examples of the three blast cell categories are shown in fig 1 Obviously transitions between the groups exist and assignment of some cells to one group or the other will be arbitrary but nevertheless, blast cell differential counts were fairly reproducible Representative

TABLE IV Skewed distribution of blast cell subtypes Typical blast cells are relatively more frequent in the marrow 300 blast cells were counted in each blood and bone marrow smear

		Distribution of blast cell subtypes		
		Typical (%)	Slightly atypical (%)	Highly atypical (%)
Case 1	Marrow	89 3	10 3	0 3
	Blood	61 0	33 7	5 3
Case 3	Marrow	72 7	24 0	3 3
	Blood	40 0	44 7	15 3

examples of labeling indices in the three blast cell types are given in table III In all cases with a morphologically heterogenous blast cell population, the typical blast cells had the highest labeling index The more atypical the blasts were, the lower was their labeling index, and the most atypical blasts were not labeled at all From these data it appeared possible that differences in the relative frequency of typical blast cells in blood and marrow might at least in part explain the observed differences between the labeling index of blood and bone marrow blast cells Blast cell differential counts done on blood and marrow smears from cases 1 2, 3, 4, and 12 confirmed this expectation In all instances the ratio typical/atypical blast cells was definitely higher in the marrow than in the blood Examples of such counts are given in table IV Since it is not possible to classify definitely every single blast cell with confidence, no attempt has been made to decide whether the skewed distribution of blast cell categories can fully account for the observed difference in blood and bone marrow labeling index

3 LABELING DATA AND CLINICAL PARAMETERS

The fraction of cells labeled with H^3 thymidine (labeling index) indicates the fraction of the cell population which was synthesizing deoxyribonucleic acid (DNA) at the time of labeling All things being equal, one would expect a positive correlation between the proliferation rate per cell and the labeling index Still keeping everything equal, a high proliferation rate per cell might be expected to have measureable consequences such as a high blast cell count in the blood, organ enlargement, and a rapid clinical course of the disease As will be discussed later, the difference between the labeling index in the bone marrow and in the blood may reflect the efficiency of a release control system in the bone marrow which tends to inhibit the emission of proliferating blast cells into the blood stream Under normal conditions very few proliferating cells escape into the circulation In the leukemic state, the ratio labeling index in marrow/labeling index in blood might reflect the efficiency of the control

inhibiting the release of cells with the capacity of further division A high ratio would mean a relatively efficient control system and a low ratio signify a profound derangement of the control It is conceivable that this could have prog nostic implications

For these reasons it was examined whether the labeling index of blood and bone marrow blast cells and the ratio between these indices were correlated with other parameters

Labeling index of blast cells in the blood

In acute leukemia (all types cases 1—20) and in myeloblastic leukemia (cases 1—11), this index did not correlate with the time elapsing from onset of symptoms to study time from study to death time from onset of symptoms to death, nor with the total blast count in the blood In order to correlate labeling indices with hepato splenomegaly a simple scor ing system was used, giving 1 point for each + of organomegaly, e g a pa tient with 1 + splenomegaly and 2 + hepatomegaly (cf tables I and II) would have a score of 3 In acute myeloblastic leukemia the score varied from 0 to 3 There was no systematic covariation between the labeling index and the degree of organ enlargement although the two lowest labeling indices were found in the two cases (nos 7 and 11) without hepato-splenomegaly and the two highest labeling indices in two cases (nos 8 and 9) with a score of 2 and 3

In chronic myeloid leukemia organ enlargement was much more pronounced than in acute leukemia (score 0—5) and the labeling index much higher However, within the group of chronic myeloid leukemia, no correlation was found be tween the labeling index and the degree of organ enlargement In myelofibrosis with hepato splenomegaly comparable to chronic myeloid leukemia (score 2—5) labeling indices were low (in the range of acute leukemia)

Labeling index in bone marrow

Data are available only on patients with acute leukemia In myeloblastic leuke mia, this index did not correlate with the degree of organ enlargement nor with the total blast count in the blood No correlation could be established with the time from onset of symptoms to study, whereas there was a significant cor relation ($p < 0.05$) between the index and the time from study to death Pa tients with a high labeling index lived longer than those with a low labeling index Similarly, there was a tendency that the time from onset of symptoms to death was longer in patients with a high labeling index than with a low index but the significance is questionable (p approx 0 1)

Ratio labeling index in marrow/labeling index in blood

In acute myeloblastic leukemia this ratio did not correlate with time from onset of symptoms to study, time from study to death, time from onset of symptoms to death nor with the total blast count in the blood The data suggest that the ratio labeling index marrow/labeling index blood is higher when hepato splenomegaly is absent then when it is present in cases 7 and 11 with no organ enlargement the ratio was 15—23, whereas it ranged from 1 8 to 5 9

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ing index) indicates the fraction of cells which was in DNA synthesis while the tracer was available In analogy to the *mitotic index*, the rate of cell production can be estimated from the labeling index if the duration of the DNA synthesis phase is known

The labeling index of normal myeloblasts is reported to range from 32—64 % (13 14, 26, 32, 35) The present data confirm and extend previous observations in vivo (23 24, 30, 31, 35) and in vitro (13, 14 26, 32) that the percentage of labeled myeloblasts is much lower in acute leukemia than in normal hemopoiesis The labeling of blast cells in acute lymphoblastic leukemia is equally low (29, 31) but data on normal human lymphoblasts are lacking Studies in which leukemic blast cells were not further classified have yielded similar results (15, 18 27)

The low labeling indices are consistent with the low mitotic indices observed in acute leukemia However before relying too heavily on these indices, some additional considerations are necessary In some tissues, considerable fluctuations in proliferative activity occur within a 24 hour period Therefore a single sample of leukemic cells usually obtained in the daytime might not adequately reflect the average proliferative activity of the tissue The problem has been studied in blast cells in the blood, no diurnal variation in labeling was found (43)

Poili et al (40 41) have questioned the applicability of thymidine as a tracer of DNA synthesis in leukemic cells on the grounds that in leukemic cells, conversion of thymidine to thymidine monophosphate may be impaired If phosphorylation of thymidine were severely curtailed in leukemic cells in general, one would expect lower grain counts in leukemic as compared with non leukemic bloods and bone marrows under otherwise similar experimental conditions This has not been our experience This of course does not preclude the existence of a fraction of leukemic cells with so little thymidine kinase activity that autoradiographically demonstrable labeling of DNA with exogenous thymidine becomes impossible However, the fact that soon after injection of H^3 thymidine in vivo the vast majority of leukemic mitoses are labeled (30) strongly indicates that H^3 thymidine labeling is a valid indicator of DNA synthesis in leukemic cells also

It appears, then, that the labeling index can be used to estimate leukemic cell proliferation rate That knowledge about the duration of DNA synthesis is also required has already been referred to Unfortunately, information on the duration of DNA synthesis in human leukemic cells as compared with normal myeloid precursors is scarce From Mauer s data (29 30) leukemic DNA synthesis would appear to last about 7—9 hours Other data suggest 3—7 hours or longer (24) Estimates of DNA synthesis *time in normal myelopoiesis vary* between 5 and 24 hours (25, 38) The most direct estimate is about 12 –14 hours (48) Thus, at the present time it is not possible to decide whether there is any difference between normal and leukemic cells with respect to duration of DNA synthesis Even if DNA synthesis periods were somewhat shorter in leukemia it appears unlikely that the difference

in the 5 cases (nos 1, 3, 4, 5, 10) in which the liver or spleen were enlarged

Comments

Until recently, attempts to characterize the growth of leukemic cells have been few (5, 9, 37) It is usually stated that leukemic proliferation is uncontrolled (17, 39, 42) and the rapidity of proliferation and frequency of mitotic figures is emphasized (7, 11, 19, 46) Current chemotherapy of leukemia is closely connected with this concept of rapid leukemic cell proliferation

When examining the validity of this general concept of blast cell proliferation in acute leukemia, it becomes clear that the problem has several aspects which must be considered separately

1 Is the cell production rate (cells formed per cell per unit time) of leukemic blast cells particularly high? To answer this question, a proliferating normal tissue is needed for reference Normal mitotable myeloid precursors would appear to be an appropriate comparison

2 Do all leukemic blast cells have the capacity to divide, or does only a fraction of the cells have this ability?

3 Are the blast cells which we observe in acute leukemia a self perpetuating cell population (i e are they stem cells in the functional sense of the term), or does the maintainance of the observable blast cell population depend on the influx of cells from an unrecognized pool of stem cells?

1 *Cell production rate of leukemic blast cells* The first study of this problem, published more than 20 years ago by Astaldi and Rivetta (2), showed that the mitotic index (fraction of cells in mitosis) was low in acute leukemia Later reports confirmed that the mitotic index of leukemic myeloblasts is lower than in normal myeloid precursors (4, 45) However, estimation of cell production rate from mitotic indices requires knowledge of the duration of mitosis (mitotic index (in %) divided by mitotic duration (in hours) equals cells produced per hour per 100 cells) This has been estimated from the accumulation of metaphase figures in bone marrow cultures to which colchicine has been added These studies suggested that leukemic blast cells proliferate more slowly than erythroblasts (1) and normal myeloid precursors (45) The results were substantiated by the subsequent observation, employing time lapse photography, that mitotic duration is considerably longer in leukemic myeloblasts than in normal myeloid precursors (6) Suggestions that leukemic cells may divide amitotically, in which case the mitotic index will not reflect the true rate of proliferation, have not been confirmed by cytophotometric studies (16)

Combined, the low mitotic index in leukemic myeloblasts in spite of prolonged mitotic duration, and the lack of evidence for amitotic cell division strongly suggests a slow cell production rate in acute myeloblastic leukemia This has been more firmly established by studies employing H^3 thymidine in vivo and in vitro Thymidine is a specific precursor of DNA Only cells which are synthesizing DNA, usually in preparation for cell division, label with H^3 thymidine (47) With a brief exposure to H^3 thymidine, the fraction of cells labeled (label

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The low labeling indices are consistent with the low mitotic indices observed in acute leukemia However before relying too heavily on these indices, some additional considerations are necessary In some tissues, considerable fluctuations in proliferative activity occur within a 24 hour period Therefore, a single sample of leukemic cells usually obtained in the daytime might not adequately reflect the average proliferative activity of the tissue The problem has been studied in blast cells in the blood no diurnal variation in labeling was found (43)

Polli et al (40 41) have questioned the applicability of thymidine as a tracer of DNA synthesis in leukemic cells on the grounds that in leukemic cells conversion of thymidine to thymidine monophosphate may be impaired If phosphorylation of thymidine were severely curtailed in leukemic cells in general, one would expect lower grain counts in leukemic as compared with non leukemic bloods and bone marrows under otherwise similar experimental conditions This has not been our experience This of course does not preclude the existence of a fraction of leukemic cells with so little thymidine kinase activity that autoradiographically demonstrable labeling of DNA with exogenous thymidine becomes impossible However the fact that soon after injection of H^3-thymidine in vivo the vast majority of leukemic mitoses are labeled (30) strongly indicates that H^3 thymidine labeling is a valid indicator of DNA synthesis in leukemic cells also

It appears, then, that the labeling index can be used to estimate leukemic cell proliferation rate That knowledge about the duration of DNA synthesis is also required has already been referred to Unfortunately, information on the duration of DNA synthesis in human leukemic cells as compared with normal myeloid precursors is scarce From Mauer s data (29 30) leukemic DNA synthesis would appear to last about 7—9 hours Other data suggest 3—7 hours or longer (24) Estimates of DNA synthesis time in normal myelopoiesis vary between 5 and 24 hours (25 38) The most direct estimate is about 12—14 hours (48) Thus at the present time it is not possible to decide whether there is any difference between normal and leukemic cells with respect to duration of DNA-synthesis Even if DNA synthesis periods were somewhat shorter in leukemia it appears unlikely that the difference

should offset the large differences in labeling index between normal (32—64 % labeled (13, 14, 26, 32, 35)) and leukemic (9—17 % labeled) myeloblasts

The combined data on mitotic index and on thymidine labeling in vitro thus point to the same conclusion in acute myeloblastic leukemia, the production rate (cells produced per cell per unit time) of myeloblasts does not exceed the production rate of mitotable myeloid cells in normal granulocytopoiesis and may well be less However, as will be discussed later, it is important to note that leukemic blast cells are probably not functionally uniform, some of them can divide but others have lost this ability A clearcut cytological distinction between proliferating and non-proliferating blast cells is difficult or impossible Mitotic and labeling indices therefore by necessity refer to blast cells in general, despite the fact that only some of them may be proliferating In normal granulocytopoiesis, on the other hand, loss of proliferative activity is associated with definite cytological changes The more precise separation of proliferating from non proliferating cells in normal granulocytopoiesis as compared with leukemia may be a major reason for the lower mitotic and labeling indices of leukemic blast cells Estimates of cell production rate from these indices refer to cells produced per cell of the mixed blast cell population Obviously, the production rate per *proliferating* blast cell will be higher Yet, the generation time (which refers to the proliferating fraction only) of leukemic myeloblasts is not particularly short, in vivo, it is estimated to range from 2 to 3 1/2 days (24) as compared with about 2 days in normal myeloid cells (10, 25) Generation times of leukemic blast cells in the order of 20 hours have also been reported (29, 30) but interpretation of the underlying data is difficult

In lymphoblastic leukemia the labeling indices are also low but a comparison with normal lymphopoiesis in man is not possible because of insufficient information Leukemic lymphoblasts are reported to have a shorter "apparent doubling time" than leukemic myeloblasts (12) but interpretation of these data in terms of proliferation rate is not possible

2 Do all leukemic blast cells have the ability to divide?

The first clue to this problem came from the observation that in acute leukemia, blast cells have a considerably lower labeling index in the blood than in the marrow (23, 24, 29, 31) (In *in vivo* studies, this refers to the initial labeling approximately 1 hour after labeling (8, 31)) Although others have failed to find this difference (26), the present study confirms that in acute leukemia, the labeling index is uniformly and distinctly lower in the blood than in the marrow

This functional difference between blast cells in different locations could be explained in several ways, single or combined

a) Cells are not released from the marrow while they are in DNA synthesis or in the latter part of the pre DNA-synthesis period (G_1-period) If cells entered the blood only during early G_1 period, most of them might again have left the blood before entering DNA-synthesis, thus explaining the

low labeling index However, after injection of H^3 thymidine in vivo, labeled blast cells appear in the blood within the first hours after labeling, and from grain count data (24) it appears that they *enter* the blood while they are in DNA synthesis or in the pre mitotic (G_2) period, contrary to the conditions required by the hypothesis

b) Cells enter the blood only late in DNA synthesis and disappear before the next DNA synthesis period is reached In this case, mitoses in the blood should be relatively numerous which they are not (24)

c) Cells are released from the marrow to the blood in the proportion DNA-synthesizing/non DNA synthesizing as they are found in the marrow Cells which are in DNA synthesis at the time of release continue DNA synthesis for some time and perhaps synthesize their full complement of DNA but no cells commence DNA synthesis while circulating in the blood Unless the blood transit time were very short as compared with DNA synthesis time, *this would result in a lower labeling index in the blood than in the marrow increasing transit time would decrease the labeling index* Actually, the blood transit time of blast cells is likely to be longer than DNA synthesis time (24) However when H^3 thymidine is added to leukemic blood at various time intervals after the blood has been drawn no significant decrease in the labeling index is observed (22) This is difficult to explain unless one assumes that cells may indeed initiate DNA synthesis in the blood Although this evidence is not sufficient to exclude the considered explanation of the low blood labeling index it makes it appear unlikely

d) Cells are released from the marrow as in (c) but cells in DNA synthesis are cleared more rapidly from the blood than those not in synthesis

e) In the preceding (a—d) it has been assumed that all blast cells are functionally uniform and explanations for the differences in labeling index in the blood and in the marrow have been sought solely in the way cells were released from marrow or blood An alternative is that two types of blast cells exist One has the capacity to divide, the other one has lost this ability Preferential release of the latter, "mature cell type from the marrow to the blood will explain the low labeling index in the blood

At present no evidence is available to help distinguish between the latter two possibilities (d—e) In normal granulocytopoiesis *proliferating precursor cells* are effectively retained in the bone marrow and only cells with no capacity for further division are released to the blood In leukemia this regulation is failing Possibility (d) involves a complete, possibility (e) a partial breakdown of normal release control Since normal homeostatic mechanisms are rarely — if ever — lost completely in disease possibility (e) is believed to be the most likely one This would seem to run contrary to a conclusion drawn from in vivo H^3 thymidine labeling, that labeled and unlabeled blast cells are emitted from the marrow approximately in the proportions in which they are present in

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the marrow (24) However, this estimate was based on a *maximum* estimate of the transit time of blast cells in the blood The true transit time may have been shorter (24), which would be compatible with a preferential release of unlabeled cells

The concept that not all cells of malignant tissue are mitotable is of recent origin (15, 23, 24, 31, 33, 34) The validity of this concept for some solid tumors has been established (33, 34) The evidence that only a fraction of leukemic blast cells proliferate will now be considered

It is often stated that all leukemic blast cells are morphologically uniform and that maturation cannot be detected by morphological standards (e g 27, 31) In part, this is correct but it does not apply to all cases of leukemia In some cases, considerable variation in size of blast cells may be found In myeloblastic leukemia, one may encounter a whole spectrum of clearly abnormal ("leukemic") cells ranging from the typical blast cells with round leptochromatic nuclei to cells with highly irregular nuclei and lacy nuclear structure giving a "monocytoid" appearance Between these extremes one finds a wide range of transitional forms with more or less irregular nuclear outline

The labeling index appears to be related to these morphological differences (21) In the present study, the labeling indices of typical, slightly and highly atypical blast cells were determined separately in all cases where this distinction could be made With no exception, the labeling index was higher in the typical than in the atypical blast cells, and the most atypical cells did not label at all Previously, karyometric measurements had shown that labeled blast cells have significantly larger nuclei than adjacent unlabeled cells (23) Recently, this observation was extended by Gavosto et al (15) who found that the labeling index of blast cells varied proportionately to the size of the cells It was felt that these differences were not adequately explained by the changes in cell size which take place during the cell cycle and it was concluded that the various size categories of blast cells represented blast cell generations of different age (15) It is an inherent difficulty of this type of classification that the average size of blast cells may vary considerably even within the same slide, depending on the thickness of the smear

The indented and sometimes segmentated nuclei of the atypical blast cells, reminiscent of nuclear segmentation in normal granulocytopoiesis, and the occasional slight chromatin condensations could be interpreted as a frustrated nuclear maturation This interpretation is supported by the low labeling index of the atypical blast cells, and the complete lack of labeling among the most atypical (most "mature") blast cells which shows that the morphological heterogeneity of leukemic blast cells is linked with a functional (ability of DNA synthesis) heterogeneity Just as normal granulocyte precursors lose their ability to divide at the metamyelocyte stage, a leukemic blast cell apparently may similarly "mature" to a stage where proliferation ceases Contrary to the thesis that leukemic cells of the blastic type "live to divide" (9), the cell has

become an end cell (It is, of course, not possible to exclude that proliferation may not be resumed if the cells were appropiately stimulated, as seems to be the case with normal small lymphocytes, but this limitation applies generally to nucleated cells, including the normal granulocyte, which at the present time are accepted as being incapable of further division) This concept of maturation processes in leukemic blast cells is in concert with the detailed cytological and cytochemical studies of Hayhoe et al (20) and with observations on cultures of human leukemic cells (36)

In all cases where the distinction could be made, typical blast cells were relatively more frequent in the marrow than in the blood thus explaining at least in part, the lower gross labeling index in the blood It cannot be decided whether the skewed distribution was due to preferential loss of 'immature' blasts from the blood or preferential release of 'mature' blasts from the marrow As referred to previously the latter possibility is the most likely one The fact that the labeling index is uniformly lower in the blood than in the marrow also in cases of acute leukemia particularly lymphoblastic leukemia with little cytological variation suggests that the existence of a non proliferating fraction is a constant phenomenon which however cannot always be recognized morphologically

Whether the relative size of the postulated non proliferating fraction remains unchanged during the course of the disease remains to be seen Among *Ehrlich* ascites tumor cells the percentage of dividing tumor cells decreases progressively with time after inoculation (3) In man, the labeling index of leukemic blast cells appears to decrease with increasing percentage of blasts in the marrow (15) This would appear to be consistent with the observations on ascites tumor cells but interpretation is difficult because data on acute and chronic leukemia were pooled (15) Serial studies on blood and bone marrow labeling during the course of acute leukemia may help to elucidate the problem

3 *Are leukemic blast cells stem cells?*

A normal hemopoietic cell line is initiated by a pool of stem cells Stem cells are self perpetuating and maintain one or more cell lines by supplying cells for differentiation This constant drain is compensated for by the stem cell s capacity for an infinite (or at least very large) number of *consecutive* divisions From the stem cell pool cells enter a multiplicative pool Here, they mature and concomitantly divide once or repeatedly However since the number of consecutive divisions of these maturing cells is small, the multiplicative pool merely serves to multiply the cell input from the stem cell pool but it cannot maintain the cell line When the cells lose their potential for further division they enter a non proliferating maturation pool from which they are eventually released as functioning cells Cell death can probably occur at any stage of a cell s development

In acute leukemia the blast cell line must necessarily be maintained by a stem cell pool This may feed directly into a non proliferating maturation pool (fig 2) or a multiplicative pool may be inserted between the stem cell and the

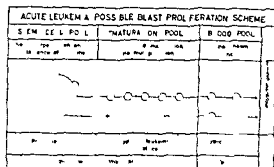


Fig 2 In this scheme the cell line is maintained by blast cells which proliferate according to a stem cell pattern Some cells lose capacity for further division and enter maturation pool where some maturation takes place Occasionally, this can be recognized cytologically giving rise to atypical blast cells (described in the text) Cells which enter the blood from the marrow (and other production sites) come preferentially from the maturation pool The association of typical' blast cells with proliferation and of atypical blast cells with non proliferation should merely be understood as approximations

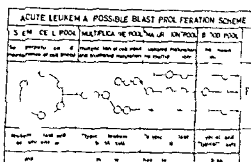


Fig 3 In this scheme the cell line is maintained either by an unrecognized stem cell or by a recognizable blast cell, located in the marrow or elsewhere cf text Some cells lose stem cell capacity of unlimited successive divisions and enter multiplicative pool Here they divide once or repeatedly and then enter maturation pool where some maturation takes place, the result of which can occasionally be recognized cytologically as atypical blast cells (described in text) Cells which enter the blood come preferentially from the maturation pool The sequence is similar to the events in normal granulocytopoiesis but maturation to a functionally useful cell is not attained and retention of proliferating cells is less efficient The association of blast cell subtypes with stage of cellular development should merely be understood as approximations

maturation pool (fig 3) According to a widespread but vague concept, reflected in the term "stem cell leukemia", the blast cells are somehow related to the stem cell(s) of normal hemopoiesis The issue is controversial (42) and factual information is lacking Whatever the derivation of the blast cells, it is important to recognize that the observable blast cells in leukemia are not necessarily stem cells, it is conceivable that they are multiplicative cells which depend on the influx from an unrecognized stem cell pool (fig 3)

If the recognizable blast cells are not stem cells, studies of the blast cells obviously will provide an incomplete picture of the entire proliferative chain from the stem cell to the 'mature' blast cell (It should be noted, however, that considerable insight into normal hemopoietic proliferation has been gained (47, 49) although the hemopoietic stem cell(s) has not been identified) In case of a stem cell pool feeding into a multiplicative pool total cell production per unit time will depend on the size of the stem cell pool, fractional release per unit time from stem cell pool to multiplicative pool, number of consecutive divisions in multiplicative pool, and the distribution of death function on the various proliferating compartments

If N^{M}_{DNA} = number of DNA synthesizing cells in multiplicative pool, and N^{S}_{DNA} = number of DNA synthesizing cells in stem cell pool, n = number of consecutive cell generations in multiplicative pool, and t^{S}_{DNA} and t^{M}_{DNA} weighted average duration of DNA synthesis in respectively stem cells and multiplicative cells,

$$N^M_{DNA} = N^S_{DNA} \times (2^n - 1) \times t^M_{DNA} / t^S_{DNA},$$

assuming stem cell production rate equals multiplicative pool influx rate, steady state, no death of proliferating cells Similarly,

$$N^M = N^S \times (2^n - 1) \times t^M_G / t^S_G$$

where N^M = total number of cells in multiplicative pool N^S total number of stem cells, and t^S_G and t^M_G weighted average generation time in respectively stem cells and multiplicative cells The duration of DNA synthesis in somatic mammalian cells varies relatively little (8) Thus the ratio between DNA-synthesizing stem cells and multiplicative cells is primarily determined by the number of consecutive multiplicative divisions, with e g 3 such divisions and no difference in duration of DNA synthesis the ratio would be 1 7 Generation time is more variable Generation time of stem cells could be as short as but could probably not be less than 10 hours, if generation time of multiplicative cells is say, 50 hours (approximately as stated for blast cells (24)), and there were 3 multiplicative divisions, the ratio between stem and multiplicative cells would be 1 35 If the ordinary dividing leukemic blast cell were a multiplicative type of cell and considering, moreover, that perhaps 50 % or more (cf the labeling indices of normal and leukemic myeloblasts referred to earlier) of all leukemic blast cells may be beyond their last division the fraction of stem cells in leukemic tissue under such circumstances might be quite inconspicuous

The relative quantitative importance of the stem cell pool and a possible multiplicative blast cell pool for total blast cell production depends on the number of consecutive multiplicative divisions, if this number is large, the multiplicative pool is the main factor in total blast cell *production*, and studies of this pool are relevant If multiplicative divisions are few, the stem cell pool must be relatively large In this case, a significant fraction of the in vitro labeled cells in the bone marrow must be stem *cells provided the* stem cells are not located elsewhere in the body, certainly, in *in vivo* studies, the kinetics of blast cells would soon be determined by the kinetic parameters of the stem cells E g, if the stem cells had a shorter generation time and a higher labeling index than the *blast cells* feeding of stem cells into the blast cell pool would be expected to result in rising blast cell labeling index with time, disproportionately high maximal blood labeling as compared with initial marrow blast labeling and shortening of blast cell generation time, as *estimated from* grain count threshold half time, with time after labeling, minor deviations of labeling indices in the directions indicated have been observed (24, 29) but may be explained in other ways as well *It* should not be overlooked, however that the observable effect of stem cell kinetics will be modified by the dilution of label with repetitive divisions

For the time being it is not possible to decide whether proliferating leukemic blast cells are stem cells multiplicative cells or both It is important to note, however that even if an unrecognized stem cell should exist there is nothing which suggests that it should be much more rapidly proliferating than the observable blast cells of acute leukemia

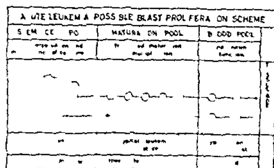


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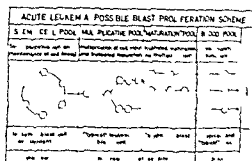


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5 Typical blast cells as a percentage of *all blast cells*, were more frequent in marrow than *in blood* This explains, at least in part, the higher labeling index of marrow blast cells

6 Various mechanisms for the skewed distribution of 'typical' blast cells between marrow and blood are considered The most likely explanation is that non proliferating blast cells are *preferentially* released from the marrow

7 Some aspects of blast cell proliferation are discussed Available data indicate that blast cell production rate (cells produced/cell/unit time) does not exceed the production rate of proliferating myeloid cells in normal granulocytopoiesis The evidence for a non proliferating fraction of blast cells is emphasized Possible schemes of observable blast cell proliferation (stem cell pattern *vs* multiplicative pattern) are considered

8 Labeling indices of blast cells in acute leukemia did not correlate with number of blast cells in blood or size of liver and spleen although there was a tendency towards higher blood labeling indices and low ratio marrow/blood labeling index when organomegaly was marked

In myeloblastic leukemia a positive correlation was found between labeling index in marrow and time from study to death

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Correlation of labeling indices with clinical parameters

No correlation could be established between labeling indices and the clinical parameters studied, with one exception in myeloblastic leukemia, the marrow labeling index was positively correlated with the duration from study to death. Whether this correlation is fortuitous or whether patients with a high labeling index fare better than those with a low index can only be determined from an independent series of observations. Offhand, patients with a low labeling index would be expected to have the better prognosis. Yet it is conceivable that chemotherapy primarily aimed at interfering with DNA-synthesis is more effective when the labeling index is high. It may be of interest to note that patient no. 1 with the highest labeling index of myeloblastic leukemias was the only one of these who went into a full remission. All patients with acute leukemia studied were treated with 6 mercaptopurine.

Labeling indices in chronic myeloid leukemia and myelofibrosis

The higher labeling index in chronic myeloid leukemia than in acute leukemia is probably related to a continuous differentiation of myeloblasts to more mature myeloid precursors in chronic leukemia; this may prevent the accumulation of non-proliferating cells in the myeloblast pool which is believed to account for the low labeling index in acute leukemia.

In myelofibrosis, the blast cells labeled to the same extent as in acute leukemia; promyelocytes and myelocytes were labeled much more frequently. Perhaps the blast cells in myelofibrosis were manifestations of a smoldering leukemic process rather than a stage in normal granulocytopoiesis. In this context it is interesting to recall the occasional termination of myelofibrosis in acute leukemia.

Summary

1. Uptake of H^3 thymidine, a labeled specific DNA-precursor, in blast cells was studied autoradiographically in 20 patients with acute leukemia, 8 with chronic myelogenous leukemia, and 5 with myelofibrosis.
2. The labeling index (fraction of cells labeled) of blast cells in the blood was low in acute leukemia, irrespective of cytologic type, and in myelofibrosis. Intermediate values were found in subacute myeloid leukemia. The highest indices were observed in chronic myelogenous leukemia.
3. In acute leukemia, the labeling index of blast cells was uniformly and distinctly lower in the blood than in the bone marrow.
4. In some cases of leukemia the blast cells could be morphologically subdivided. Typical blast cells had higher labeling index than "slightly atypical" blast cells. "Highly atypical" blast cells did not label at all. These types are believed to represent various stages of blast cell maturation. The highly atypical blast cell appears to have lost its ability to divide and is believed to be an end product of blast cell proliferation.

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Free Fatty Acids of Plasma During Spinal Anaesthesia in Man[1]

By

Dag Hallberg and Lars Oro[2]

The adrenergic neurohormones, noradrenaline and adrenaline, stimulate the mobilization of free fatty acids (FFA) from adipose tissue *in vitro* as well as *in vivo* (7 10 11 13) The role of the catecholamines and the sympathetic nervous system in the mobilization of FFA to plasma is also indicated by studies in which sympathetic blocking agents have been used Administration of the sympathetic ganglionic blocking agent hexamethonium to anaesthetized fasting dogs decreased the mobilization into and the level of FFA in blood plasma (13 14) Chemical sympathetic blockade inhibited the rise of the FFA level during conditions with increased sympathetic activity e g during mental stress (3) cold stress (22) hypoglycaemia (1 9 26) and experimental trauma (6) The rise in the FFA level induced by electrical stimulation in CNS in anaesthetized dogs was prevented by the administration of a sympathetic ganglionic blocking agent (20)

Sympathetic blockade can also be produced by injection of local anaesthetics, as in spinal anaesthesia Beside the well known changes in the cardiovascular system that may occur during spinal anaesthesia (8 16) a decreased oxygen consumption has been observed (24) This finding suggests that spinal anaesthesia also may cause metabolic changes

In this investigation the level of FFA in arterial blood plasma was studied during spinal anaesthesia in man The plasma levels of glycerol and glucose were followed simultaneously

Material and methods

Nineteen male patients 17 to 55 years old were studied after they had fasted at least 15 hours They attended the surgical clinic because of inguinal herniae Otherwise they appeared healthy as judged from the history and the routine clinical and laboratory

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[1] A preliminary report was given before the VIII Congress of the Scandinavian Society of Anaesthesiologists in Turku Finland, August 1964

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By

DAG HALLBERG and LARS ORO[2]

The adrenergic neurohormones noradrenaline and adrenaline stimulate the mobilization of free fatty acids (FFA) from adipose tissue *in vitro* as well as *in vivo* (7 10 11 13) The role of the catechol amines and the sympathetic nervous system in the mobilization of FFA to plasma is also indicated by studies in which sympathetic blocking agents have been used Administration of the sympathetic ganglionic blocking agent hexamethonium to anaesthetized fasting dogs decreased the mobilization into and the level of FFA in blood plasma (13, 14) Chemical sympathetic blockade inhibited the rise of the FFA level during conditions with increased sympathetic activity e g during mental stress (3) cold stress (22, hypoglycaemia (1 9 26) and experimental trauma (6) The rise in the FFA level induced by electrical stimulation in CNS in anaesthetized dogs was prevented by the administration of a sympathetic ganglionic blocking agent (20

Sympathetic blockade can also be produced by injection of local anaesthetics as in spinal anaesthesia Beside the well known changes in the cardiovascular system that may occur during spinal anaesthesia (8 16) a decreased oxygen consumption has been observed (24) This finding suggests that spinal anaesthesia also may cause metabolic changes

In this investigation the level of FFA in arterial blood plasma was studied during spinal anaesthesia in man The plasma levels of glycerol and glucose were followed simultaneously

Material and methods

Nineteen male patients 17 to 55 years old were studied after they had fasted at least 15 hours They attended the surgical clinic because of inguinal herniae Otherwise they appeared healthy as judged from the history and the routine clinical and laboratory

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TABLE I Changes in the systolic blood pressure (B P) and pulse rate (P R) during spinal anaes was injected Mean ± S E of mean

Spinal anaesthesia	Duration of complete muscle paralysis (min)		Mean level when methylthionine was injected
High (Thl—Th3)	60—90 (n=6)	B P (mm Hg)	128 ±10
		P R (beats/min)	72 ± 4
	10—60 (n=3)	B P (mm Hg)	135 ± 7
		P R (beats/min)	76 ±12
	0—10 (n=5)	B P (mm Hg)	135 ± 8
		P R (beats/min)	74 ± 6
Low (Th 10—L 1)	60—90 (n=5)	B P (mm Hg)	143 ± 5
		P R (beats/min)	76 ± 7

[1] Indicates statistical significance $p < 0.05$

examination No premedication was given before the operations which were performed about 9 a m A teflon catheter was inserted into one brachial artery, after production of local anaesthesia using prilocaine (= Citanest®, ASTRA) The subjects then rested between 20 and 10 minutes before the spinal anaesthesia The blood pressure was measured over the brachial artery using a sphygmomanometer cuff in the usual manner and feeling for the systolic radial pulse (at an accuracy of 5 mm Hg)

Spinal anaesthesia

A Pitkin needle was introduced into the spinal canal, between L 2 and L 3, until liquor started to drip from the needle Methylthionine (1 5 ml = 15 mg Tetracain grave[b], ACO) was then injected into the subdural space The operation table was tilted to produce high or low spinal anaesthesia

High spinal anaesthesia

In 14 subjects the dermatomes up to the level of Th 1—Th 3 were insensitive for pain and cold after 10 to 20 minutes This effect lasted during 60 to 90 minutes The skeletal muscles in the lower part of the body were paralyzed to a varying degree The paralysis was defined as complete when no voluntary movement in the legs could be produced The patients were divided into three groups according to the duration of the complete paralysis (60—90 10—60 and 0—10 minutes respectively)

thesia in man The figures are calculated on the individual changes from the level when methylionine

Mean change (min after injection of methylionine)							
10	20	30	40	50	60	90	120
[1] −30 ±9	[1] −27 ±8	[1] −23 ±8	−22 ±9	−20 ±9	−19 ±9	−15 ±6	−10 ±6
−4 ±2	−6 ±3	−7 ±4	−7 ±4	−6 ±3	−5 ±3	−6 ±3	−5 ±2
−8 ±4	−22 ±13	−32 ±18	−27 ±15	−20 ±12	−18 ±17	−13 ±12	−9 ±9
−8 ±6	−12 ±9	−15 ±13	−15 ±13	−13 ±13	−11 ±14	−9 ±8	−9 ±6
−29 ±11	[1] −43 ±11	[1] −43 ±13	[1] −41 ±11	[1] −40 ±10	[1] −32 ±10	[1] −27 ±8	−20 ±6
−2 ±2	−6 ±3	−9 ±5	−10 ±5	−10 ±5	−11 ±6	−10 ±6	−6 ±5
−2 ±3	−2 ±3	−6 ±6	−14 ±8	−13 ±5	−11 ±5	−9 ±4	−6 ±3
4 ±4	2 ±3	0 ±3	−3 ±4	−5 ±5	−7 ±6	−7 ±7	−9 ±7

Low spinal anaesthesia

In 5 subjects the dermatomes up to the level of Th 10—L 1 were insensitive to pain and cold after 10 to 20 minutes and during 60 to 90 minutes The legs were completely paralyzed between 60 and 90 minutes

All subjects received 700—1 000 ml saline intravenously during the study The operations started 25—30 minutes after the injection of methylionine and lasted 25 to 40 minutes

Analysis

Arterial blood samples were withdrawn into heparinized syringes and immediately centrifuged No heparin was injected into the subjects. The FFA were determined according to Dole (7) Enzymatic methods were used for analysis of glycerol (27) and blood glucose (19) Statistical analysis was performed as recommended by Snedecor (25)

Results

High spinal anaesthesia with complete muscle paralysis 60 to 90 minutes

There were 6 subjects in this group Ten minutes after the injection of methylionine the mean systolic blood pressure had decreased from 128 to 98 mm Hg ($p < 0.05$) (fig 1 and table I) The blood pressure then tended to return to the initial level No significant change in the

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anaesthesia in man The figures are calculated on the individual changes from the level when methyltio-

Mean change (min after injection of methyltionine)							
10	20	30	40	50	60	90	120
−0 12 ±0 08	*−0 26 ±0 04	*−0 28 ±0 05	*−0 31 ±0 04	*−0 26 ±0 05	−0 17 ±0 08	0 00 ±0 11	0 19 ±0 15
−0 014 ±0 005	*−0 018 ±0 004	*−0 024 ±0 006	*−0 020 ±0 007		0 003 ±0 011	0 014 ±0 006	*0 029 ±0 007
−0 07 ±0 04	−0 13 ±0 04	−0 17 ±0 08	−0 14 ±0 12	−0 06 ±0 10	0 05 ±0 15	0 17 ±0 13	0 33 ±0 15
−0 008 ±0 014	−0 011 ±0 014	−0 013 ±0 014	−0 018 ±0 007		0 015 ±0 007	0 015 ±0 019	*0 042 ±0 003
0 10 ±0 04	0 16 ±0 11	0 22 ±0 11	*0 32 ±0 07	*0 42 ±0 08	*0 46 ±0 07	*0 39 ±0 09	*0 31 ±0 10
0 010 ±0 005	*0 027 ±0 010	0 031 ±0 009	*0 037 ±0 011		*0 037 ±0 008	*0 032 ±0 008	*0 021 ±0 004
0 18 ±0 04	0 21 ±0 02	0 18 ±0 08	0 20 ±0 10	*0 19 ±0 06	*0 21 ±0 02	0 14 ±0 06	0 09 ±0 08
0 017 ±0 002	*0 013 ±0 003	0 014 ±0 007	0 006 ±0 007		0 025 ±0 014	0 012 ±0 015	0 010 ±0 019

No significant change in the pulse rate was observed (table I) Thirty minutes after the injection of methyltionine the mean FFA level had decreased from 0 86 to 0 69 mEq/l ($p > 0.05$) (table II) Ninety minutes later the FFA had increased to a mean level of 1 19 mEq/l The glycerol level followed almost parallel with the FFA level (fig 2) Fourty minutes after the injection of methyltionine the mean level had decreased from 0 097 to 0 079 mMol/l ($p > 0.05$) (table II) The blood glucose concentration was almost unchanged at a mean level of 90 mg/100 ml (fig 2)

High spinal anaesthesia with complete muscle paralysis 0 to 10 minutes

There were 5 subjects in this group In 3 of them the paralysis was never complete Thirty minutes after the injection of methyltionine the mean systolic blood pressure had decreased from 135 to 92 mm Hg ($p < 0.05$) (fig 3 and table I) No significant change in the pulse rate occurred from the initial mean level of 74 beats per minute (fig 3 and table I) The mean FFA level increased during the spinal anaesthesia (fig 3) The maximal rise from 0 66 to 1 12 mEq/l was observed 60 minutes after the injection of

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High (Th1—Th3)	60—90 (n=6)	FFA mEq/l	0 70 ±0 02
		Glycerol mMol/l	0 084 ±0 008
	10—60 (n=3)	FFA mEq/l	0 86 ±0 17
		Glycerol mMol/l	0 097 ±0 015
	0—10 (n=5)	FFA mEq/l	0 66 ±0 07
		Glycerol mMol/l	0 085 ±0 009
Low (Th 10—L1)	60—90 (n=5)	FFA mEq/l	0 73 ±0 13
		Glycerol mMol/l	0 094 ±0 015

[1] Indicates statistical significance $p < 0.05$
[2] Indicates statistical significance $p < 0.01$
[3] Indicates statistical significance $p < 0.001$

pulse rate, from the initial mean frequency of 72 beats per minute, was observed (fig 1 and table I) The mean FFA level in blood plasma decreased after methylthionine had been injected and reached the lowest level after 40 minutes (fig 1 and table II) The mean fall was from 0 70 to 0 39 mEq/l ($p < 0.001$) The mean FFA concentration then successively increased during the following 80 minutes and reached a level of 0 89 mEq/l The glycerol level followed the same pattern as the FFA level (fig 1 and table II) Thirty minutes after the injection of methylthionine the mean glycerol level had decreased from 0 084 to 0 060 mMol/l ($p < 0.05$) After 120 minutes the mean glycerol level had increased above the initial level to 0 113 mMol/l ($p < 0.01$) The blood glucose concentration was unchanged during the anaesthesia at a mean level of 88 mg/100 ml (fig 1)

High spinal anaesthesia with complete muscle paralysis 10 to 60 minutes

There were 3 subjects in this group Thirty minutes after the injection of methylthionine the mean systolic blood pressure had decreased from 135 to 103 mm Hg ($p > 0.05$) (fig 2 and table I)

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10	20	30	40	50	60	90	120
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−0 07 ±0 04	−0 13 ±0 04	−0 17 ±0 08	−0 14 ±0 12	−0 06 ±0 10	0 05 ±0 15	0 17 ±0 13	0 33 ±0 15
−0 008 ±0 015	−0 011 ±0 014	−0 013 ±0 014	−0 018 ±0 007		0 015 ±0 007	0 015 ±0 019	*0 042 ±0 003
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High spinal anaesthesia with complete muscle paralysis 10 to 60 minutes

There were 3 subjects in this group Thirty minutes after the injection of methylionine the mean systolic blood pressure had decreased from 135 to 103 mm Hg ($p > 0.05$) (fig 2 and table I)

methylnonine ($p < 0.01$) (table II) The FFA level then slowly decreased to 0 97 mEq/l The glycerol level followed the same pattern as the FFA concentration, from the initial mean level of 0 085 mMol/l (fig 3 and table II) The blood glucose concentration did not change significantly from the initial mean level of 90 mg/100 ml (fig 3)

Low spinal anaesthesia with complete muscle paralysis 60 to 90 minutes

In the 5 subjects with low spinal anaesthesia no significant changes in the blood pressure or the pulse rate were observed from the initial mean levels of 143 mm Hg and 76 beats per minute, respectively (fig 4 and table I) The mean FFA concentration increased from 0 73 to about 0 93 mEq/l (fig 4) The rise was statistically significant after 10 20 50 and 60 minutes respectively (table II) The mean glycerol level appeared to follow the FFA concentration, from the initial mean level of 0 094 mMol/l (fig 4) The rise in glycerol was statistically significant after 10 and 20 minutes (table II)

The blood glucose concentration remained almost unchanged at a mean level of 80 mg/100 ml (fig 4)

Discussion

The anaesthetic and paralytic effect of spinal anaesthesia is explained by a blockade of the impulses in the nerve roots to and from the spinal cord (8, 12 23) The sympathetic nerves which leave the spinal cord between Th 1 and L 2 (thoracicolumbar outflow) (23) may also be affected Accordingly there was a fall in blood pressure without increase in heart rate in all groups with high spinal anaesthesia (Th 1—Th 3) but not in the group with low spinal anaesthesia (Th 10—L 1) In the subjects with high spinal anaesthesia an increased FFA level in plasma was observed in those subjects showing a short lasting paralysis of the legs This indicated that a symphathetic vasomotor blockade is not always accompanied by a decreased FFA level in blood plasma On the other hand a decreased FFA level was a consistent finding in those subjects showing a long lasting paralysis of the legs This finding suggests that the FFA level in blood plasma is influenced by nerves from the spinal cord, which together with the nerves to skeletal muscles, are affected by spinal anaesthesia

It is not known if the changes in the level of FFA in blood plasma were due to variations in the rate of mobilization from adipose tissue or to variations in the fractional turnover rate of FFA in plasma Circulatory changes in different organs, such as the liver the skeletal muscles and the kidneys may influence the rate of removal of FFA from blood plasma and therefore also the fractional turnover rate However, under various conditions changes in the FFA concentration in blood plasma are mainly caused by changes in the FFA production rate (for extended discussion and references see Oro and Wallenberg (20) Furthermore, in the groups with high spinal anaesthesia increased as well as decreased FFA levels were observed In all of them there was a fall in blood pressure without significant change in pulse rate Evidently the FFA changes were not related to these circulatory parameters

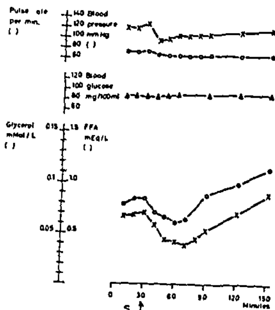


Fig 1 Free fatty acids (FFA) and glycerol in arterial blood plasma, blood glucose, systolic blood pressure and pulse rate during high spinal anaesthesia (Th 1—Th 3) with complete muscle paralysis in the legs during 60 to 90 minutes The mean changes from studies on 6 subjects are given Methylnonine was injected into the sub dural space at S Operations for inguinal herniae were started 25 to 30 minutes later and lasted between 25 and 40 minutes

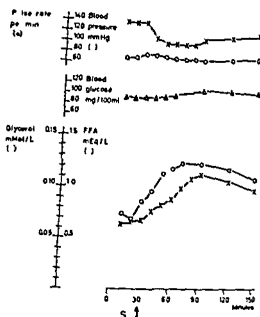


Fig 3 Free fatty acids (FFA) and glycerol in arterial blood plasma blood glucose, systolic blood pressure and pulse rate during high spinal anaesthesia with complete muscle paralysis of the legs during 0 to 10 minutes The mean changes from studies on 5 subjects are given

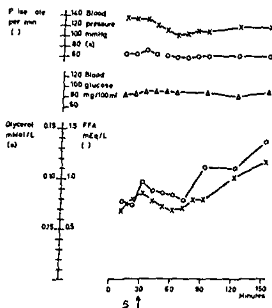


Fig 2 Free fatty acids (FFA) and glycerol in arterial blood plasma, blood glucose systolic blood pressure and pulse rate during high spinal anaesthesia with complete muscle paralysis of the legs during 10 to 60 minutes The mean changes from studies on 3 subjects are given

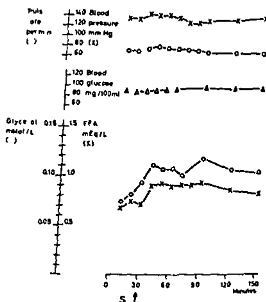


Fig 4 Free fatty acids (FFA) and glycerol in arterial blood plasma blood glucose systolic blood pressure and pulse rate during low spinal anaesthesia with complete muscle paralysis during 60 to 90 minutes The mean changes from studies on 5 subjects are given

studied during spinal anaesthesia in 19 men who were operated upon for inguinal hernia

In 14 men *high spinal anaesthesia* was produced with absent pain and cold appreciation up to Th 1—Th 3 during 60 to 90 minutes The systolic blood pressure decreased without change in pulse rate The legs were completely paralyzed during 0 to 90 minutes In one group (n = 6) in which the paralysis lasted *60 to 90 minutes* the FFA and glycerol levels decreased significantly The levels did not change significantly in a second group (n = 3) in which the paralysis lasted *10 to 60 minutes* In a third group (n = 5) in which the paralysis lasted *0 to 10 minutes* the levels of FFA and glycerol increased significantly

In 5 men *low spinal anaesthesia* was produced with anaesthesia up to Th 10—L 1 during 60 to 90 minutes and with complete paralysis of the legs during 60 to 90 minutes No significant changes in the blood pressure or heart rate were observed The levels of FFA and glycerol increased

No significant changes in the blood glucose levels were observed

The results suggest that there are nerves from the spinal cord which influence upon the FFA mobilization from adipose tissue It appears possible that these nerves leave the spinal cord between Th 1—Th 3 and Th 10—L 1, and *that* they are separate from the sympathetic vasomotor nerves of importance for blood pressure regulation

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Glycerol is released from adipose tissue when its triglycerides are hydrolyzed. The glycerol level in plasma was followed here, as evidence is available indicating that the concentration of glycerol in plasma reflects the lipolytic activity in adipose tissue (4, 5, 15). The glycerol level was found to follow the FFA level in all patients studied. These results suggest that the FFA changes observed here were mainly caused by variations in lipolysis in adipose tissue with changes in the rate of mobilization of FFA into blood plasma.

Lewis and Page (17) recently found that after high spinal cord transection in dogs, the levels of FFA were low and the blood glucose levels were unchanged. In this study a decrease in the FFA level was only observed during high and never during low spinal anaesthesia. It is therefore possible that there are nerves, of importance for the mobilization of FFA, which leave the spinal cord from the same region as the thoracicolumbar sympathetic outflow (Th 1 to L 2).

The secretion of catecholamines from the adrenal glands is considered to be influenced by sympathetic preganglionic fibres leaving the spinal cord from Th 10 to Th 12 (23). It is noteworthy in this connection that Luft and von Euler (18) found that patients with disturbed sympathetic vasomotor control (postural hypotension) also showed a deficiency in liberating noradrenaline and adrenaline. The adrenal catecholamine secretion may therefore have been inhibited in all groups with high spinal anaesthesia, as the blood pressure fall without increase in heart rate indicated a disturbed sympathetic vasomotor function.

The possible role of the adrenal catecholamine secretion for the mobilization of FFA has been studied by several investigators (2, 9, 13, 14, 20). They have found that the FFA mobilization can occur during many conditions with increased sympathetic nervous activity in adrenalectomized as well as in intact man and animals. The non-adrenal part of the sympathetic nervous system thus seems to be of major importance for FFA mobilization.

It was recently found (21) that "orthostatic stress", e.g. tilting, produced a rise in the FFA level even in patients with postural hypotension. Supramedullary stimulations in anaesthetized dogs sometimes elevated the levels of FFA and glycerol in blood plasma without changing the heart rate or blood pressure (20). From these observations as well as from the present study it appears possible that the mobilization of FFA into plasma is influenced by nerves which are separate from the sympathetic nerves of importance for the cardiovascular control.

The plasma FFA level has been reported to be increased by physical and mental stress as well as "orthostatic stress" (3, 6, 14, 22). Thus, the hernia operations may have been a form of stress which explains the rise in the FFA level which was observed in most subjects at the end of the anaesthetic period. If so, the stimuli causing this rise were blocked during different time intervals in those subjects showing a decreased FFA level.

Summary

The levels of free fatty acids (FFA) and glycerol in arterial blood plasma were

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Organ Antibodies in Disseminated Lupus Erythematosus

By

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Disseminated lupus erythematosus (D L E) is a disease which affects many organs and tissues producing patterns which vary not only from patient to patient but also within the same patient during the course of the disease Assuming that D L E is a real clinical entity its most characteristic feature is its lack of organ specificity

It is believed that autoimmune mechanisms are involved in the pathogenesis of D L E This view is supported by the presence of anti nuclear and anti cytoplasmic antibodies in sera from patients with this disease The organ antibodies most often encountered in D L E are non organ specific like the disease itself However organ specific antibodies such as thyroid and gastric antibodies have also been demonstrated in sera from patients with D L E According to previously published papers the occurrence of specific thyroid complement fixing antibody in D L E sera varies from 2 % (8) to 27 % (21) The occurrence of thyroglobulin antibody has been reported in 13 % (21) 16 % (17) and 20 % (12), and parietal cell antibody in 2 % of the cases (18)

In the present paper the results are presented of organ serological reactions performed on 29 sera from patients with D L E The sera were examined for the presence of specific thyroid, adrenal, salivary and parietal cell antibodies, and for non organ specific complement fixing antibody non organ specific precipitins and for anti nuclear factor The findings are compared to those found in sera from 38 cases of Hashimoto s disease 27 of non tuberculous Addison s disease 25 of pernicious anemia 21 of Sjogren s syndrome and 29 control cases

Methods

Complement fixing microsomal thyroid antibody was demonstrated as described by Roitt and Doniach (19) In order to secure the specificity microsome fractions of toxic goitres were used as antigens instead of crude extracts

Thyro-cytotoxic factor was demonstrated as described by Irvine (14) and by Pulvertaft et al (18) and standardized as described by Halberg (10)

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Fig 5 Fluorescence of parietal cells of gastric glands.

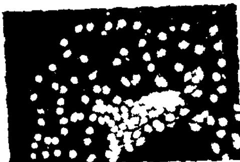


Fig 6 Nuclear fluorescence in a thyroid cell culture



Fig 7 Fluorescing mitotic figures in HeLa cell culture

Anti nuclear factor was demonstrated by an immune fluorescence technique on un fixed sections of various organs Sections of thyroids were most convenient for the pur pose although the results were sometimes difficult to read especially when testing sera containing antibody against the cytoplasm of the follicles In such cases the test was done on thyroid cells grown in ring chambers as described by Halberg (11) (fig 6) When thyroid epithelial cells have been grown for four or five days these cells lose their microsomal antigen but the nuclear antigens seem to remain intact HeLa cells i e an old strain of carcinoma cells may also be used for this purpose In cultures of this kind fluorescent mitotic figures may be seen (fig 7)

Non-organ specific complement fixing antibody was demonstrated as described by Gajdusek (9) and by Hijmans et al (12) High concentrations of crude extracts of toxic or atoxic goitres were used as antigen In order to ensure that the antibody thus demonstrated was not the specific microsomal thyroid anti body only such results were considered sig nificant in which no reaction was obtained using a thyroid microsome fraction as an antigen

Non-organ specific precipitins were dem onstrated as described by Jones (16) and by Anderson et al (2) The tests were performed by a double diffusion technique in agar gel placed on slides High concentra tions of thyroid extracts were used as antigen Precipitin reactions caused by high titers of thyroglobulin antibody were ruled out by the fact that no precipitin reaction was obtained when using purified thyroglobulin instead of thyroid extract by the lack of an identity reaction when co-precipitating the sera con taining non-organ specific precipitins with Hashimoto sera containing high titers of thyroglobulin antibody and by the fact that in all cases the positive sera contained no thyroglobulin antibody or only low titers of thyroglobulin antibody as demonstrated by the TRC method

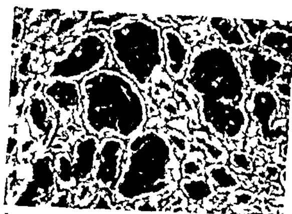


Fig 1 Fluorescence of cytoplasm of thyroid follicular epithelium

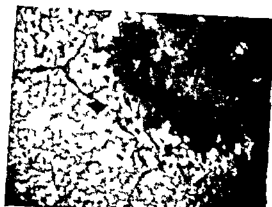


Fig 3 Fluorescence of cytoplasm of adrenal cortical cells Border between cortex and medulla

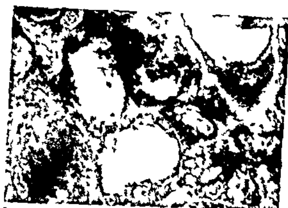


Fig 2 CA 2 antibody Diffuse fluorescence of colloid in thyroid follicles

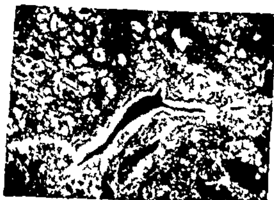


Fig 4 Fluorescence of nuclei and of cytoplasm of the cells of salivary excretory ducts

The sera were tested for the presence of antibody causing cytoplasmic fluorescence of the thyroid follicular epithelial cells by an immune fluorescence technique on unfixed sections of toxic goitres as described by Holborrow et al (13) (fig 1)

CA 2 antibody was demonstrated as described by Balfour et al (3) by an immune fluorescence technique on methanol fixed sections of thyroid tissue Since thyroglobulin antibody may cause difficulties in reading the results only sera with a thyroglobulin antibody titer of less than 250 (determined by the TRC v i) have been examined for the presence of the CA 2 antibody (3) (fig 2)

Thyroglobulin antibody was demonstrated by means of thyroglobulin coated sheep cells (TRC) from Bourroughs Wellcome & Co

Antibody against adrenal cortex was demonstrated by an immune fluorescence technique as described by Blizzard et al (5) Unfixed sections of guenon adrenals were used for the test because it was found that the results are identical when using human and guenon adrenals The guenon adrenals have a convenient size so that each slice is a cross section of marrow and cortex The border between marrow and cortex is well defined and in positive tests there is a striking contrast between the dark marrow and the bright fluorescence of the cortex (fig 3)

Antibody against the excretory ducts of the salivary glands was demonstrated by means of an immune fluorescence technique on unfixed sections of salivary tissue as described by Bertram and Halberg (4) (fig 4)

By approximately the same technique antibody against the gastric parietal cells was demonstrated as described by Irvine (15) (fig 5)

TABLE III Thyroid antibodies

	Sera	Complement fixing antibody	Follicular cytoplasmatic fluorescence	Cytotoxic factor	Thyroglobulin antibody	CA — 2 antibody
D L E	29	3	3	3	6	7/28
Control	29	0	0	0	5	9/29
Hashimoto s thyroiditis	38	35	35	35	35	5/7

striking feature of the tables is the rare occurrence of organ specific antibodies in sera from patients with D L E compared with the organ specific diseases in which organ specific antibodies are frequently found In those few cases in which organ specific antibodies were found in sera from patients with D L E the titers were often high and the fluorescence was often bright and compared well with the findings in the organ specific diseases Even though few organ specific antibodies were found in sera from D L E the occurrence was more common than in the control sera The organ specific antibody found most frequently in sera from patients with D L E was the antibody against the excretory ducts of the salivary glands It was found in 7 of 29 cases Two of the 7 sera came from D L E patients with Sjogren s syndrome whereas no signs of Sjogren s syndrome were found in the other 5 patients Specific thyroid microsomal complement fixing antibody thyrocytotoxic factor and the antibody causing cytoplasmic fluorescence of the follicular epithelium were found in the same three sera One of these sera also contained thyroglobulin antibody in a rather high titer (25 000) The other D L E sera containing thyroglobulin antibody had low titers of 250 or less The same was true for the control sera

TABLE IV Antibody against adrenal cortex

	Sera	Antibody
D L E	29	0
Control	29	0
Addison s disease	27	19

TABLE V Parietal cell antibody

	Sera	Antibody
D L.E.	29	1
Control	29	0
Pernicious anemia	25	19

TABLE VI Antibody against salivary excretory ducts

	Sera	Antibody
D L E	29	7
Control	29	0
Sjogren s syndrome	21	12

TABLE I Occurrence of lesions in 29 cases of D L E

Arthropathy	25
Cirrhosis of the liver	13
Nephropathy	4
Anemia	18
Hemolytic disease	4
Leukopenia (< 2,500)	9
Thrombocytopenia (< 100,000)	11
Skin lesion (discoid and disseminated L E)	7
Scleroderma	4
Pericarditis	3
Pleurisy	3
Myositis verified by biopsy	9
Complete alopecia	1
Raynaud s syndrome	4
Fever	9
Sjogren s syndrome	2
Pernicious anemia	1
Addison s disease	1
Positive Wasserman reaction	2

TABLE II Ages of 29 patients with D L E

Age	No of patients
10—19	3
20—29	6
30—39	2
40—49	4
50—59	7
60—69	5
70—79	2

Material

Sera from 29 patients with D L F were included in the material All of the patients presented pronounced L E cell phenomena 27 of the 29 patients had hypergammaglobulinemia The lesions presented by these 29 cases of D L E are stated in table I All the patients presented at least two of these lesions, most of them had more than two lesions

All of the patients were women The ages of the patients are stated in table II The graph shows two peaks In this materia D L E was found particularly often in youn women in their 20 s and in elderly women in their 50 s

The control material comprised 29 women the ages of whom closely corresponded to the patients with D L E The control cases were hospital patients with gastric or duodenal ulcers, chronic constipation, coronary occlusion and psychiatric disorders In each case the ESR was less than 10 mm/hr

The 38 cases of Hashimoto s disease were all verified by biopsy

The 25 cases of pernicious anemia were diagnosed by the finding of megaloblastic marrows, low levels of serum B 12 and by Schilling tests

The 27 cases of Addison's disease were diagnosed by characteristic clinical findings, values of urinary excretion of 17 hydroxy corticosteroids, subnormal levels of plasma cortisol and pathological ACTH stimulation tests Patients with tuberculosis and adrenal calcifications were excluded from the material

Twenty one cases of Sjogren's syndrome were included in the material In 5 cases only was the diagnosis verified by biopsy, the rest of the diagnoses being based on the results of sialography, sialometry, Schirmer s test swelling of the salivary glands and changes of the oral mucous membranes Eight cases presented signs of lesions of the salivary and lachrymal glands only The remainder of the patients had also rheumatoid arthritis cirrhosis of the liver or thrombocytopenia i e lesions characteristic of D L E Two patients with Sjogren s syndrome had disseminated symptoms and positive L E cell tests Consequently they were included in the D L E material Since several transitional forms between D L E and Sjogren s syndrome were found the limit between the two groups had to be arbitrary

Results

The occurrence of organ specific antibodies is stated in tables III to IV A

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of the 29 sera from patients with D L E but it was found in none of the control sera One D L E serum contained both specific thyroid complement fixing antibody and non organ specific complement fixing antibody This could be demonstrated by absorption experiments and by block titration Non-organ specific precipitin was found in only 4 D L E sera and in none of the control sera

Non-organ specific antibodies were rarely found in sera from patients with organ specific diseases It is particularly remarkable that anti nuclear factor was demonstrated in only one of the 38 Hashimoto sera though it was looked for in nuclei in sections from several different tissues and in cell cultures Three of the Addison sera and 2 of the sera from patients with pernicious anaemia contained anti nuclear factor Non organ specific complement fixing antibody and non organ specific precipitin were not found in sera from patients with Hashimoto s disease Addisons s disease and pernicious anemia

The Sjogren sera which often contained an organ specific antibody, namely an antibody against the cytoplasm of the salivary excretory ducts, frequently also contained non organ specific antibodies Anti nuclear factor was found in 11 non organ specific complement fixing antibody in 9 and non-organ specific precipitin in one of the 21 sera

Discussion

Clinically diseases of a possible auto immune pathogenesis may be divided up into three groups 1) organ specific diseases in which only one organ or organ system seems to be affected, 2) non-organ specific diseases in which a variety of organs or systems of organs are affected at the same time in varying patterns, and 3) a group of diseases in which a lesion of one organ is the predominating feature but in which a combination with other organ lesions is often encountered Hashimoto s disease, Addison s disease and pernicious anemia are examples of organ specific diseases In these diseases the thyroid, the adrenal cortex, and the parietal cells respectively are affected Disseminated lupus erythematosus is an example of a non-organ specific disease, and Sjogren s syndrome represents the third group This disease is predominated by chronic sialoadenitis and dacryoadenitis, but it is often associated with rheumatoid arthritis, cirrhosis of the liver, and other lesions

As previously suggested by Roitt and Doniach (20) the organ serological findings of these three groups of diseases come in patterns with a striking similarity to the clinical findings This suggestion corresponds well with our findings according to which sera from patients with the diseases of the first group contained almost exclusively organ specific antibodies mainly directed against antigens specific to the affected organs However clinical and serological overlaps between Hashimoto s disease and pernicious anemia (8) and between Addison s disease and thyroid disease (6) are known Sera from patients with D L E contained mainly non-organ specific antibodies and the few organ specific antibodies found corresponded generally poorly with the clinical findings As far as the third group is con

TABLE VII Non organ specific antibodies

	Sera	Anti nuclear factor	Complement fixing antibody	Precipitin
D L E	29	28	18	3
Control	29	7	0	0
Hashimoto s thyroiditis	38	1	0	0
Pernicious anemia	25	2	0	0
Addison s disease	27	3	0	0
Sjogren s syndrome	21	11	9	1

in which thyroglobulin antibody was found All the D L L patients were euthyroid, and none of those in whose sera thyroid antibodies were found had goitres Two of the patients whose sera contained complement fixing thyroid antibody, had tracer tests with I[131] performed which were normal, in particular the PBI[131] after 48 hours was not elevated in either case One patient with D L E had antibody against parietal cells in her serum However she had no signs of pernicious anemia, she had a normal serum B_{12} and normal acidity of her gastric juice This patient was one of those who had complement fixing thyroid antibody in her serum Moreover, her serum was the only one of the D L E sera which did not contain anti nuclear factor Neither did it contain the non-organ specific complement fixing antibody This serum differed from the other D L E sera by containing only specific antibodies The patient was a young woman of 25 years with rheumatoid arthritis, leukopenia, severe Raynaud's phenomenon, myositis by biopsy, hypergammaglobulinemia and pronounced L E cell phenomenon None of the 29 sera from patients with D L E contained antibody against adrenal cortex, but one patient had Addison's disease One patient had pernicious anemia, but her serum did not contain antibody against parietal cells

In contrast to the rare occurrence of organ specific antibodies in sera from patients with D L E organ specific antibodies were frequently found in sera from patients with organ specific diseases All of the 38 cases of Hashimoto's disease had at least one thyroid antibody in their sera Nineteen of the 25 patients with pernicious anemia had antibody against parietal cells, 19 of the 27 patients with Addison's disease had antibody against adrenal cortex, and 12 o the 21 patients with Sjogren s syndrome had antibody against the excretory ducts of salivary tissue in their sera

Non organ specific antibodies were very frequent findings in sera from patients with D L E All but one of the sera contained anti nuclear factor which was a fairly common occurrence also in the control sera since it was found in 7 out of 29 cases Non organ specific complement fixing antibody was found in 18

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cerned, Sjögren's syndrome represents a condition in which both organ specific and non organ specific antibodies were frequently found

It would be tempting trying to place some other diseases of a possible autoimmune origin in the three groups mentioned Clinically, some cases of rheumatoid arthritis and cirrhosis of the liver fit in the same group as Sjogren's syndrome since one lesion is the predominant feature but a combination with other lesions characteristic of D L E is common It is already known that anti nuclear factor and non organ specific complement fixing antibody are often found in sera from patients with these conditions (1, 7, 9), but antibodies against antigens specific of joints and liver have not yet been found Essential thrombocytopenia and some cases of acquired hemolytic disease might fit into the same group which may eventually turn out to contain diseases with lesions characteristic of D L E but without other signs that make it possible to establish a diagnosis of D L E

Summary

Sera from 29 cases of D L E were tested for the presence of specific thyroid, adrenal, parietal cell, and salivary antibodies, and for the presence of non-organ specific antibodies, i e, anti-nuclear factor, non organ specific complement fixing antibody and non-organ specific precipitin Organ specific antibodies were rarely found whereas non-organ specific antibodies were frequently found The results were compared with the findings in sera from patients with chronic thyroiditis, Addison's disease, and pernicious anemia in which organ specific antibodies were common whereas non organ specific antibodies were rare Moreover the results were compared with those found in sera from patients with Sjogren's syndrome in which both organ specific antibodies (against the cytoplasm of the excretory ducts of the salivary tissue) and non-organ specific antibodies were frequently found

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A Case of Mesothelioma Pericardii

By

E Lopes Cardozo and J F Saltet

A mesothelioma formerly also referred to as endothelioma or endothelial carcinoma is a tumour arising from the layer of cells lining the serous cavities, which have developed from the splanchnocele of the embryo These cells are called mesothelial cells in order to distinguish them from the cells derived from the ectoderm and the entoderm Mesotheliomas are mostly found in the pleura those of the peritoneum and the pericardium are much less frequent They may develop at any age, recently Kauffman (10) reported on cases in children

As early as 1875 Marchiafava (13) described a pericardial tumour which he called an endothelioma In 1936 Geschickter (6) suggested the term mesothelioma for such tumours In 1947 Reals (17) reviewed 22 cases described in the literature in 1953 Dawe (4) collected 25 cases The clinical features of the mesothelioma pericardii are very well illustrated by a case reported by Thomas et al (21) In 1960 both Forest (5) and Yraola (26) added some cases to the literature Mairot (12) collecting also the European literature in his thesis mentioned 68 cases including 3 observed by himself of which he gave a detailed description In 1962 Gonin (8) published a paper discussing Mairot s 3 cases to which he added one case seen by himself One of these cases however should be classified in their opinion as a thymoma Gonin (8) also mentioned one case in which the pericardial tumour was resected at the pedicle This patient was given post operative radiotherapy and was still alive five years after the operation Saltet and Van der Esch (18) described a case with the clinical symptoms of coronary thrombosis, autopsy however revealed a minute pericardial mesothelioma In the latter case death was probably caused by haemopericardium, brought about by a haemorrhage from the tumour partly due to anticoagulant therapy

In the Netherlands the first case was reported by ten Seldam (19) who called it carcinoma of the pericardium

Submitted for publication March 3 1965





F g 4 F g 5

F gs 4 and 5 Mesothelioma of the par etal and visceral perica d um in the lo er r ght part normal m ocardial tissue. F g 4 H E. 50 × (reduced 1/2 ×) F g 5 The fissures in the tumour tissue can be recognized 18ɔ × (reduced 1/2 ×)

lar ve ns the cyanos s the marked dilatation and shape of the heart on the X ray and finally the alternans in the ECG all ind cated pericard al effus on Pleural puncture on the left s de was performed on March 14 and 120 ml of serous fluid was aspirated This puncture d d not produce any mpro ement Per cardial puncture was done on March 16 the needle now be ng nserted deeper at the same site This time haemorrhagic fluid 700 ml) was asp rated whi h brought mme d ate rel ef The congest on in the jugular eins decreased considerably the heart vas smaller on the next X ray

Soon however per cardial effus on re turned and puncture was repeated on March 20 25 and 29 Ne ertheless the patient s con d on became rap dly worse attacks of extreme dyspnoea succeeded each other at shorter nter vals oxygen fa led to bring adequate relief The patient d ed on March 31 1964 probably as a result of card ac tamponade Permission for necropsy was obta ned

Labo a ory find ng

Blood ESR 23 mm/1 hour Hb 16 1 g/100 ml erythrocytes 4 100 000 leukocytes 12 500 d fferent al count normal Blood urea 37 5 mg 100 ml creatin ne 0 69 mg/100 ml choles erol 183 mg/100 ml b l rub n 0 5 mg 100 ml thymol turb d ty 0 4 U alkaline phosphatase 20 King Armstrong U SGOT 32 U SGPT 23 U

Urine albumin 1 °₀ glucose — urobulin + b lirub n — sediment 6—10 erythrocytes 10—15 leukocytes some casts

Cytolog cal findings in the pleural exudate mostly lymphocytes and patholog cal ep the lial cells also cells of malignant type s ngle or in groups large nucleus/cytoplasm rat o cytoplasma varying from light to dark blue on G emsa staining nuclei with coarse chromat n structure and clearly perceptible nucleoli (f g 2) After acridine orange stain ng and exam nat on by blue fluorescent microscopy the large tumour cells showed orange fluorescence of their cytoplasm and nucleoli (fig 3)

The same cytological pattern mixed with many erythrocytes was found in the peri card al fluid

Anatomical find ngs

Pericard um and ep cardium were uniformly nodular greyish white and thickened to 4 mm The pericard um conta ned about 500 ml haemorrhagic flu d The heart was com pressed w thout any further abnormal ties and we ghed 300 g

In the left parietal and visceral pleura only a few small flat greyish wh te tumours were found this pleural cavity contained about 800 ml of a bloody fluid No abnormalities in the right visceral and parietal pleura

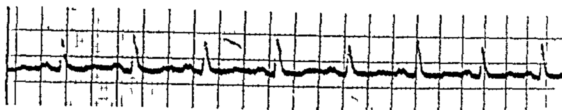


Fig 1 Electric alternans in lead 2

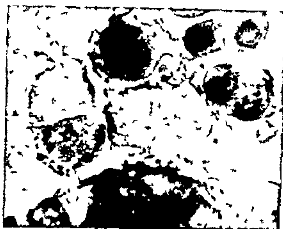


Fig 2 Giemsa stain of the aspirated pericardial fluid 1,000 × (reduced 2/3 ×)



Fig 3 Acridine orange stain of the pericardial fluid seen by blue fluorescence 1 000 × (reduced 2/3 ×)

Godwin (7) in particular has given a detailed description of the characteristics of metastasized mesothelioma The following case of a mesothelioma arising from the pericardium also had already extensive metastases

Case report

A 54 year old woman came to our outpatient department on March 11, 1964 She complained of dyspnoea and palpitations, which she had had for the past three weeks and which had become more serious during the last week Nevertheless she had continued to work until that day

On examination her pulse was rapid, irregular and unequal (144/min), with a deficit of 20 B P 140/90 The central venous pressure, as measured at the jugular veins was R + 2 There was slight cyanosis, no oedema The patient was dyspnoeic even at rest

On percussion the heart extended up to 2 cm to the right of the sternum, the left heart border could not be defined as a result of a pleural effusion The cardiac sounds were weak There was normal vesicular breathing, no rales

The liver was about 5 cm enlarged and did not pulsate The ECG showed rapid fibrillation with little amplitude of the QRS complex, and rounding of the ST segment in various leads

An X ray of the chest showed a spherical heart enlarged to the right and left, congested hili and pleural effusion left of the heart The patient was hospitalized immediately after examination

Intensive digitalis therapy was instituted, and 250 mg pronestyl was given by mouth every 6 hours The next morning the rhythm of the heart was regular at 104 The ECG now showed an electric alternans (fig 1), there was no pulsus alternans During the night the patients suffered a brief episode of severe dyspnoea The temperature was subfebrile between 37—38 C (98 6—100 4 F) The severe congestion of the jugu

lymphocytes are usually found, which in the past has often suggested tuberculosis Usually it does not seem possible to differentiate mesotheliomas from other types of malignant tumours on the basis of mere cytological findings

An early recurrence of the effusion after puncture, seems to be common in all cases recorded so far (21) Usually the symptoms are of comparatively short duration, and the disease is fatal within a year Treatment is mostly ineffective, X ray therapy has occasionally yielded a definite cure (1)

No positive results have been observed with cytostatic drugs Surgical treatment may be considered in localized pleural mesotheliomas, and has also been performed in a pericardial mesothelioma (8)

B Pathogenesis and pathological findings

Mesothelium may be described as epithelium functionally differentiated from connective tissue It is a layer of cells lining the coelomic cavity from which arise the pleural pericardial and peritoneal cavities Tissue cultures of mesothelium by Maximow (14) yielded mainly fibroblasts with some epithelial structures Pellissier and Ouary (15) made cultures of embryonic mesenchyme which produced fibroblasts as well as some epithelium like cells

Tumours arising from the mesothelium may also give rise to both cell types Stout and Murray (20) made cultures of tissue from a fibrous mesothelioma and observed a phase of epithelial growth while connective tissue developed in a later stage

It has been suggested that mesotheliomas of the pleura may result from an earlier pleurisy In the period 1954—1963, pleural mesothelioma was diagnosed 35 times in Rotterdam in 14261 necropsy specimens In only 4 cases had there been a history of pleurisy

Brown and Johnson (2) assumed a past history of pleurisy in 3 of their cases Nevertheless the rarity of these tumours as compared with the high rate of adhesive pleurisy in necropsy findings, suggests that pleurisy as an underlying cause is most unlikely It is also remarkable that peritoneal mesothelioma is extremely rare in man Only 4 such cases were revealed by the Rotterdam necropsies during the same period a negligible number as compared with the many cases of peritonitis Recent reports from South Africa by Wagner (23 24) and Thomson (22) have indicated asbestosis as a possible cause of pleural mesothelioma In a retrospective study of 15 mesotheliomas they demonstrated the presence of asbestos in 9 of the lung tissue specimens which were still available The relationship was also demonstrated experimentally in rats Determination of the hyaluronic acid (produced by the mesothelium) was valuable in these investigations It may be that also some peritoneal and pericardial mesotheliomas result from the transport of asbestos particles in the lymphatics

Over a 19 year period we found 8 cases of pericardial mesothelioma, including the one mentioned above They included 4 men and 4 women aged 42—80 The data yielded no evidence of pericarditis in any of these patients

Both lungs contained several peripheral emboli, there was an infarcted area in the right lung The emboli had been derived from a thrombosis of the right femoral vein The lymph glands in the hilus of the lung were small and greyish black The liver contained numerous greyish-white tumours up to about 2 cm in diameter, and weighed 2,100 g Throughout the bone marrow of the spine were greyish white spots In the cortex of the right kidney two small greyish spots were observed No abnormalities were discovered in other organs No permission had been given to dissect the brain

Microscopical findings

Circumscribed pseudotubular foci with relatively few cells were found in the parietal and visceral pericardium, embedded in fibrous connective tissue (figs 4 and 5) The focal lesions were made up of polymorphous vesicular cells containing nuclei of varying chromatin density, with sporadic mitosis They did not form real tubules, rather fissures Invasion in the heart muscle was rare The same applied to the pleural tumours, there was only slight invasion of the lungs A similar aspect was seen in the tumour tissue of the liver and bone marrow, which could be regarded as haematogenous metastases Laguesse staining, as well as P A S and mucicarmine staining, was negative The white spots in the right kidney were due to ischaemic necrosis resulting from thrombosis, which had probably originated in the left heart The septum atriorum was completely closed

The immediate cause of death must have been a combination of chronic heart tamponade and multiple pulmonary embolism

Discussion

1 Clinical symptoms

In many respects the symptoms resemble those of exudative pericarditis dyspnoea, cough, oppression and pain in the cardiac region, and some bloody sputum Congestion of the jugular veins is an early phenomenon, followed in a later stage by enlargement of the liver and oedema of the legs Pleural effusion, as seen in our case, is by no means rare, as the tumour may infiltrate into the pleura The heart is considerably enlarged, usually the heart beat can not be felt, and the cardiac sounds are very weak Pericardial friction may be present Paradoxical pulse as well as an alternating pulse have been observed

On the X ray the heart is generally enlarged to a balloon shape, fluoroscopy shows the absence of pulsations After pericardial puncture and the injection of some air, the pericardium may sometimes be seen as a shell of irregular thickness (3)

Rhythmic disturbances are often seen in metastasized pericardial tumours In our case there was paroxysmal fibrillation The ECG often shows symptoms of hypoxia, with a depressed ST segment or negative T wave Electric alternans is a common phenomenon, which may be accompanied by an alternating pulse In the Netherlands electric alternans has been described by Piso (16) and van Hees (9)

Electric alternans is seen as a sign of cardiac compression in exudative pericarditis, which sometimes may be caused by tumour growth (McGregor (11), Burch (3)) but it may also occur in diffuse myocardial lesions, and occasionally in tachycardia

Cytological studies of the usually haemorrhagic fluid obtained by puncture are of considerable value in the diagnosis of pericardial tumours Andolf (1) first demonstrated tumour cells in this way Besides tumour cells many

us The 4 cases diagnosed as mesothelio mas from peritoneal origin did not show any metastases Reals et al (17) have reported a case of pericardial mesothelio ma with metastasis and invasion of the lungs and haematogenous metastases in the kidneys

Lymphogenous metastasis has seldom been observed except in the hilar glands of the lung, but often the pos ibility of permeation cannot be excluded

Summary

Report on a primary mesothelioma of the pericardium in a 54 year old woman Cytological studies of the aspired exudate enabled us to establish the diagnosis of pericardial tumour before death Not withstanding repeated punctures the course of the disease was rapidly fatal, with signs of heart tamponade

At autopsy metastases were found in the pleura the liver and the bone mar row

The clinical and pathological features are discussed

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Among our own patients, puncture was performed in 5 cases of pleural mesothelioma and in only 1 case of pericardial mesothelioma (described in this paper) In 4 cases a mesothelioma was suggested from cytological findings (and on the fact that no primary tumour could be detected elsewhere) Pleural biopsy was performed 8 times, in 4 cases a mesothelioma was diagnosed from histological findings, in 4 cases an adenocarcinoma was tentatively diagnosed

It is obvious that the diagnosis is difficult, even from histological data, because the possibility of an undetected carcinoma elsewhere can never be definitely ruled out In the latest edition of his "Pathology of tumours", Willis (25) still rejects the possibility of mesothelioma as a primary tumour Consequently a thorough investigation, which should include the bronchial tree, thyroid gland, breasts, pancreas, gall bladder and internal sexual organs (prostate!), is of the utmost importance Dawe et al (4) distinguished pericardial mesotheliomas into epithelial, fibrous and mixed types In our opinion this holds good for all mesotheliomas The mixed type particularly is often typical and clearly shows the dual potentialities of the mesenchyme All cases mentioned by us, the present case included, were of the mixed malignant type

The mesothelial cells often line a fissure like cavity of irregular shape, sometimes they form a most remarkable tubular structure, like that of an adenocarcinoma In differentiating the tubular, or rather the pseudotubular type of mesothelioma from adenocarcinoma, haematoxylin and some other methods of staining are of value Laguesse's silver impregnation will stain the basement membrane If it is present, it indicates a carcinoma rather than a mesothelioma, as normal mesothelium is developed directly from the underlying stroma of connective tissue

Mucicarmine staining will demonstrate mucus in the vacuoles if there are any, thus indicating carcinoma of the digestive tract rather than mesothelioma.

Positive P A S staining after amylase treatment demonstrates that the vacuoles do not contain glycogen, but real mucus, which indicates carcinoma rather than mesothelioma

Finally it is important to demonstrate hyaluronic acid in the tissue of the mesothelioma It is of considerable clinical value that hyaluronic acid may also be found in the pleural exudate

By this method Wagner et al (24) studied 16 pleural mesotheliomas, in 5 cases they found an increased hyaluronic acid level of the exudate Their technique was as follows

Pleural exudate was diluted with 2.5 parts of water and acidified with 30 % acetic acid to obtain an acetic acid concentration of 0.2 % Hyaluronic acid then forms a kind of clot This does not happen if the exudate has first been treated with hyaluronidase at 38° C for 30 minutes

We do not know if hyaluronic acid has ever been determined in pericardial exudate Theoretically it should yield results similar to those obtained in pleural exudate

Haematogenous metastasis of pleural and pericardial mesotheliomas was found in about one third of all cases studied by

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Non-synchronized Direct Current Countershock

Conversion of Atrial Fibrillation and Subsequent Electrocardiographic Findings

By

E K Waris, T M Scheinin, K E Kreus, J Salokannel and B M Scheinin

In 1962 Lown et al (6 7) published their observations on the use of synchronized direct current shock in certain cardiac arrhythmias chronic atrial fibrillation could be effectively converted to sinus rhythm in this way The method has aroused interest, and according to reports subsequently published sinus rhythm has been successfully restored in 70—92 per cent of the cases (1 4, 8 9 10 11 19) Complications have been rare and they have not been serious, though recently severe arrhythmias (3, 11 14) and even cases with fatal outcome (13) have been reported

The heart is supposed to tolerate the countershock without ventricular fibrillation if the shock is not delivered in the vulnerable period of the cardiac cycle (6) The present report describes the authors experience to date on the use of non synchronized direct current countershock in the treatment of chronic atrial fibrillation For a few days following defibrillation, electrocardiographic changes were noted fairly frequently, mainly as changes of the ST segment and T wave As far as is known, relatively little attention has hitherto been devoted to them (4 12, 19)

Material and methods

The series consisted of 36 patients 24 men and 12 women Their ages ranged from 35 to 77 the mean age being 57 years. The duration of atrial fibrillation determined on purely objective criteria was less than 6 months in 21 cases, 6 months to 2 years in 9 cases and over 2 years in 6 cases The true duration of atrial fibrillation had probably been longer in many cases though this was impossible to determine from the medical history Three patients had had embolic episodes

All the patients were on digitalis and some who were in cardiac failure were given diuretics After a test dose they were given quinidine for a minimum of 3 days (1 2 g/day) Twenty-four patients were given anti-coagulant treatment for at least three weeks prior to the attempted conversion

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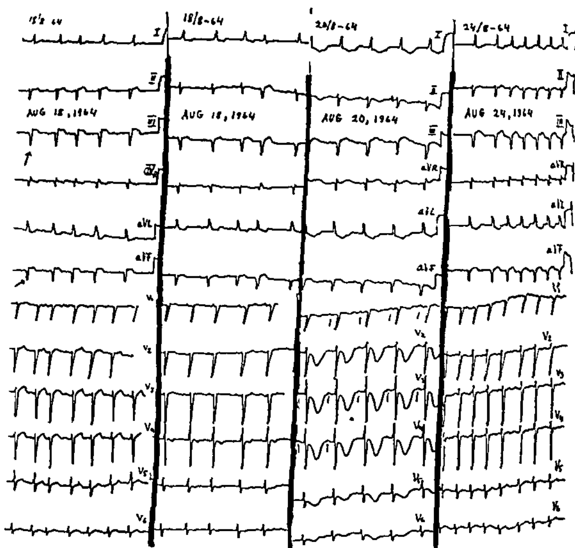


Fig 1 Patient A S female 58 with an old inferior myocardial infarction and treated thyrotoxicosis Aug 18 after defibrillation normal sinus rhythm depression of ST segment in leads III and aVF depression or inversion of T wave in leads I II V_3—V_6 Aug 20 ECG shows further inversion of T wave especially in leads I aVL V_2—V_6 An U wave is seen e g in leads I II III aVR aVL aVF and V_1 Aug 24 reversion to atrial fibrillation T wave inversions disappearing

Premedication given before defibrillation was 50 mg pethidine and 0 5 mg atropine Conversion was performed in the operating theatre, where precautions could be taken for possible resuscitation The patient first inhaled pure oxygen for about 5 minutes and was then put under general anaesthesia with 100—250 mg sodium pentobarbital injected intravenously After induction the patient was given 30—50 mg succinylcholine and ventilated with pure oxygen without intubation If the attempt at conversion was prolonged anaesthesia was continued with a 50 50 mixture of nitrous oxide and oxygen

Countershocks were given with a direct current defibrillator without synchronization device (Corbin Farnsworth Palo Alto Calif) A 100 watt second discharge was given initially and subsequently if required the countershocks were 200 and 400 watt seconds These 400 watt second shocks were repeated either until normal sinus rhythm was achieved or until further attempts were considered futile for the time being The electrodes were

placed either according to Lown (7), so that one was at the cardiac apex and the other to the right of the sternum in the 2nd intercostal space ("oblique position"), or in a transverse position (1) with one electrode on the median axillary line at the left 4th intercostal space and the other at the 3rd intercostal space at the right beside the sternum

In 7 cases the conversion failed and defibrillation was attempted again after continued medication The total of attempted defibrillation was thus 43 on 36 patients

After defibrillation the patients remained in the hospital under observation for at least 4 days and came for follow up examinations after 1 3 and 6 months During the first 3 months the quinidine treatment was continued with a daily dose of 0 8—1 2 g Digoxin Diuretics glyceryl trinitrate etc were given to patients as required



Fig 2 The same patient as in fig 1 The day after defibrillation short periods of subsequent ventricular ectopic beats occurred

Results

Primary results

Conversion to normal sinus rhythm was considered to have been achieved on 38 occasions (88 %) In most cases there were various ectopic extrasystoles immediately after the conversion, but they usually disappeared in the first 10 minutes There were no severe complications, such as ventricular fibrillation or embolism SGOT changes were noted in one

TABLE I Etiology of the heart disease and primary result of defibrillation

Etiology	No of cases	Heart rhythm after defibrillation			
		Immediately		Next day	
		NSR	AF	NSR	AF
Rheumatic heart disease					
Mitral	12	12	0	12	0
Aortic	2	2	0	2	0
Coronary disease	18	14	4	11	7
Hypertensive heart disease	7	6	1	5	2
Thyrotoxic heart disease	4	4	0	2	2
Total	43	38 (88°)	5	32 (77 %)	11

NSR = Normal sinus rhythm AF = Atrial fibrillation.

TABLE II Heart volume and left atrial size in relation to immediate result of D.C. countershock

Cardiac, left atrial size[1]	Heart: No of cases in NSR	Heart: No of cases in AF	Left atrium: No of cases in NSR	Left atrium: No of cases in AF
Normal	3	0	5	0
Large	14	3	23	5
Giant	21	2	10	0

Heart volume normal = relative heart volume up to 500 ml/sq m in men 450 ml/sq m in women large = from 451 or 501 to 700 ml/sq m, giant > 700 ml/sq m.

[1] Left atrial size was estimated arbitrarily

TABLE III Number of countershocks needed for conversion to normal sinus rhythm

No of shocks	1	2	3	4	5	6	7	8	9	
Energy level	100	200	400	400	400	400	400	400	400	Total
Converted to NSR	2	6	18	6	3	1	1	0	1	38
Remained in AF						2	1	1	1	5

case, but the ECG, however revealed no changes indicative of myocardial damage Another patient, after a conversion that had required 7 countershocks suffered pain over the whole thoracic area on the second post-conversion day, there was a deep depression of the T wave (fig 1) and short attacks of ventricular ectopic beats (fig 2) but no increase in SGOT or sedimentation rate The etiology of atrial fibrillation seemed to influence the immediate results (table I) Normal sinus rhythm returned to all patients suffering from rheumatic heart disease while atrial fibrillation in cases of coronary artery disease proved more resistant The other etiological subgroups were small, for atrial fibrillation on the basis of thyrotoxicosis the results were not promising despite euthyroidism at the moment of conversion

Defibrillation was successful on all patients under the age of 50, but failed in one-third of the older patients (7 21) Here the primary disease causing the atrial fibrillation was a noteworthy factor for the younger patients suffered mainly from rheumatic heart disease and the older from coronary artery disease. Advanced age did not necessarily predispose to a poor result for normal sinus rhythm was restored to 3 of the 4 patients aged over 70 years

The success of the primary defibrillation attempt was independent of the duration of atrial fibrillation It also appeared that success of conversion did not depend on the total heart

TABLE IV Duration of normal sinus rhythm after conversion

	Primary results		Follow up results			
	Imme diately	Next day	4th day	1 month	3 months	6 months
Remained in NSR	38	32	29	22	17	11
Reverted to AF	[1](5)	6	9	15	19	22
Unknown	0	0	0	1	2	5

[1] Unsuccessful defibrillation attempts

TABLE V Number of direct current countershocks in each conversion attempt in relation to immediate and later results

No of D C shocks	No of cases	Primary conversion	Reverted to AF in 1 day	4 days	1 month	3 months	Total
1–3	26	26	4	0	4	2	10
4–9	17	12	2	3	2	2	9

volume or on the left atrial size (table II) The energy of the countershock required for conversion was also independent of the size of the heart or atrium

Usually (in 74 %) 1—4 countershocks were required to achieve normal sinus rhythm In most cases the third shock, 400 watt seconds was successful, since the preceding discharges employed were only 100 and 200 watt seconds (table III) As a rule, attempts beyond the fourth or fifth countershock gave no result

The electrodes were in transverse position in 86 countershocks of which 21 (24 %) resulted in sinus rhythm and oblique during 66 discharges of which 17 (26 %) resulted in conversion Seven discharges were given in other positions The location of the electrodes therefore did not affect the outcome of attempted defibrillation even though both main groups contained resistant cases in which a change in the position of the electrodes produced normal sinus rhythm

Duration of induced sinus rhythm

As yet there are late results for only a number of the patients (table IV) Of the 38 conversions atrial fibrillation returned the following day to 6 patients and during the short period of postconversion hospitalization it returned to another 3 patients A month after conversion 60 % and 3 months after conver-

TABLE VI Number of countershocks in each conversion attempt in relation to electrocardiographic changes and other complications after treatment

No of shocks	No of cases	Electrocardiographic changes: T and ST depression, U wave, ST elevation	I and II° heart block	Ectopic nodal atrial, ventr beats	Elevation of: SGOT	Leuc. count	Burns Gr I
1–3	26	8	1	3		2	
4–9	17	10	1	3	1	1	3

sion 47 %, of the defibrillated patients who had come for a follow up examination had preserved the normal sinus rhythm After 6 months, 33 % out of examined 33 patients have retained the normal sinus rhythm

Defibrillation was again attempted because of reversion of atrial fibrillation or primary failure, in 7 cases Two of these patients retained atrial fibrillation unchanged, in 3 it returned the following day, in 1 by the fourth day and in the seventh patient within a month The duration of the sinus rhythm attained was the shorter the more numerous the countershocks required for conversion (table V) If 1—3 countershocks were needed to attain sinus rhythm, atrial fibrillation returned within 3 months in 10 patients out of 26 with primary conversion, whereas of the 12 patients to whom 4—9 shocks had to be given, 9 had a reversion of atrial fibrillation within this period

At the follow-up examination after 3 months the sinus rhythm was found to have persisted in 7 patients with rheumatic heart disease out of the 11 examined For the group with arteriosclerotic heart disease the figures were 6 out of 17

In the youngest age group, those under 50 years of age, the sinus rhythm has persisted up to the 3 month follow-up examination in 6 patients out of 7 so far examined, in the age group of 51—65 years in 7 patients out of 16, and in the group aged over 65 in 4 patients out of 10 Total heart volume or left atrial size did not seem to affect the constancy of the sinus rhythm achieved

Electrocardiographic findings after defibrillation

Several changes were noted in ECGs taken the day after both successful and unsuccessful attempts at conversion (table VI) Inversion of the T wave and/or depression of the ST segment were seen particularly frequently (see graphs), in connection with a total of 18 out of the 43 attempted conversions These changes were reversible and disappeared usually within 4 days or at least were absent after 1 month

A comparison of the number of countershocks given and the later electrocardiographic changes revealed that depression of the T wave and the ST segment was more frequent among the patients who had had several discharges

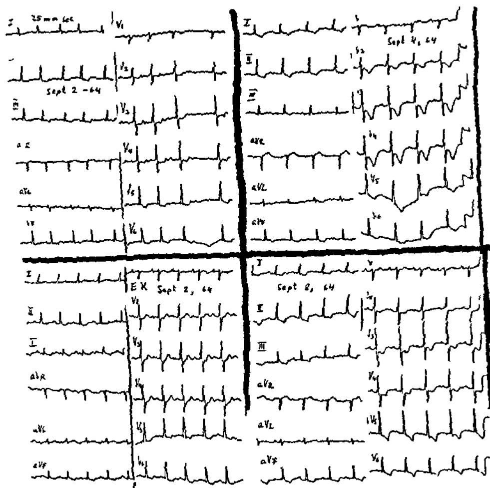


Fig 3 Patient E K female 48 with rheumatic heart disease mitral incompetence and atrial fibrillation (Sept 2) The same day after defibrillation a P wave appeared regularly with a prolonged PR time Two days later *inversion* of T wave is seen with depression of ST segment in leads V_1—V Six days after defibrillation the same changes are still present though decreasing Sinus rhythm continues

(table VI) On the other hand, they also occured in patients who had 1—2 countershocks and failed to occur in 4 of the 5 patients who had 7—9 discharges These changes were seen in patients of all etiology groups and as often in the patients who were given diuretics as in those who did not get these drugs

Ectopic ventricular and atrial extra systoles occured in the majority of cases immediately after defibrillation Sometimes conduction time also showed instability during the first few minutes after the countershocks Arrhythmias developed later in 6 cases *1* patient (A S fig 2) had a short period of

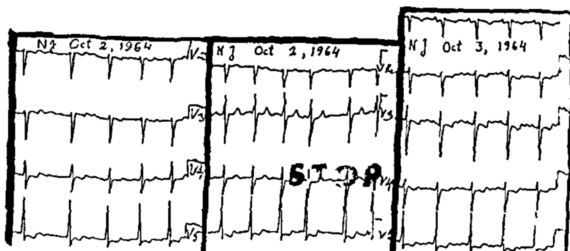


Fig 4 Patient N J, male, 62 with coronary heart disease Depression of ST segment and T wave after defibrillation (Before, after and next day)

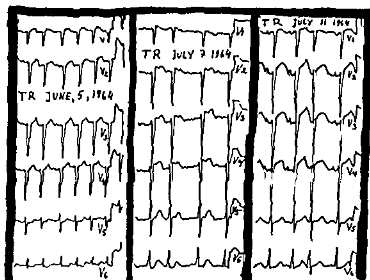


Fig 5 Patient T R, female 57, with old anterior myocardial infarction and atrial fibrillation (June 5 and July 7) The day after defibrillation (July 11) sinus rhythm with instability of PR interval and elevation of ST segment

ventricular ectopic beats and others atrial or nodal rhythm Two patients had 1st or 2nd degree heart block

Other observations

Most of the patients felt well after defibrillation, and a sensation of some kind of euphoric well-being was characteristic of many The effect of defibrillation on cardiac compensation was assessed on the basis of the following criteria change in circulation time, lung and liver stasis and oedema elsewhere No facilities for determining cardiac output were available

On the basis of this criteria, the situation on the days following defibrillation as compared with that prior to defibrillation was definitely better for 12 patients, slightly better for 9 patients, and no definite change for 17 patients

TABLE VII Results in DC counter shock therapy in atrial fibrillation

Authors	No of cases	Conversion to ASR	Conversion (%)
Lown et al. (7)	85	78	92
Oram and Davies (11)	100	84	84
Killip (4)	46	40	87
Cullhed et al (1)	27	19	70
Varnauskas et al (19)	77	53	71
Miller (9)	20	18	90
Present series	36	31	84

Discussion

Synchronized direct current counter shock is considered by many a quick and easy method in the treatment of cardiac arrhythmias safe and relatively reliable (1, 4 7 8 9 11 12 19) In the present series a total of 160 direct current countershocks without synchronization, were given to 36 patients No attempts were made to avoid the vulnerable period of the cardiac cycle during the initial portion of the T wave No ventricular fibrillation occurred and the incidence of the conversion of atrial fibrillation corresponded to figures so far reported *in the literature (table VII)*

In the present series attention was attracted by the frequent though transient electrocardiographic disorders which occurred after attempted conversion The commonest were the inversion of the T wave and depression of the ST segment Little attention has up to now been devoted to these changes according to the information available however they have been less frequent than in the present series Killip (4) describes one case of this kind in his series of 66 patients while Sussman et al (18) reported on a case in which a transient rise of the ST segment suggestive of myocardial changes, also occurred without accompanying changes in SGOT level or other laboratory tests Oram and Davies (11) in their series of 100 patients reported the occurrence of injury current in 12 cases Most authors have indicated that 1–3 or at the most 4 countershocks were given in connection with each attempted conversion In the present material, this number was exceeded in 11 cases and it appeared with some exceptions that the electrocardiographic changes were commonest among those who had received multiple shocks (table VI) *It was also found that* an attempt at conversion prolonged beyond the fourth countershock was seldom successful Experience suggested that repeated attempts were useless and even harmful

In most cases the depression of the ST segment or inversion of the T wave and the appearance or growth of the U wave were like those seen in hypokalemia (2) Repeated depolarisations may expel potassium from myocardial cells and produce electrolyte disorders No serum electrolyte disorders have been noted (19) nor was it possible for us to

reverse the electrocardiographic changes by potassium infusion

These changes seemed to have had no relationship to the etiology of the heart disease nor to the diuretic drugs given before defibrillation to some patients

Other electrocardiographic changes, such as ventricular and atrial extrasystoles, short attacks of ventricular ectopic beats (in 1 case), 1st or 2nd degree heart block or instability of conduction time were seen in the present series, and occurred on a scale largely similar to that reported by earlier authors (3, 4, 8, 11, 19)

SGOT was found to be elevated in only one patient, who had received the very high number of 9 countershocks and yet showed no electrocardiographic changes Killip (4) reported elevated SGOT in 3 cases and Cullhed et al (1) in 7 out of 27 cases For all patients except one the latter connected this elevation with the local skin and muscle changes at the sites of the electrodes Many authors, however, have not found any SGOT rises (7, 8 10, 11, 19) A case has also been described in which the patient, because of repeated attacks of ventricular fibrillation, was given a total of 140 direct current countershocks in 69 hours, yet no signs of injury to the myocardium were noted on autopsy (5) Recently, Rabbino et al (13) reported on 3 cases of fatal ventricular fibrillation following attempted conversion of digitalis induced arrhythmias

The present incidence of adverse effects in connection with direct current defibrillation was therefore perhaps higher than that indicated in earlier reports, but the changes were not considered sufficiently severe as to bar the future use of the method Nonetheless, treatment of non hospitalized patients must be considered inadvisable until the significance of the electrocardiographic changes has been made clear The absence of a synchronization device does not explain their occurrence, for the results obtained with the synchronized direct current defibrillator currently in our use do not seem to differ from those obtained with the equipment used for the present study More countershocks per conversion and higher energy amounts than has generally been described in the literature were required, perhaps partly because the present series contained many aged patients with coronary artery disease or degenerative heart failure, and partly because only those cases of atrial fibrillation resistant to quinidine and digitalis therapy were treated with direct current defibrillation

The necessity for general anaesthesia has been much discussed, and some authors are of the opinion that direct current countershocks, especially the first shock, can be given either with sedation only (11) or without any anaesthesia (8, 17) The present series included some cases in which the first countershock was given without anaesthesia and the subsequent attempts were carried out under general anaesthesia Because of the unpleasant sensations felt during defibrillation, these patients unhesitatingly preferred anaesthesia For reasons of safety, too, light anaesthesia may be useful since preparedness for resuscitation is then improved (15, 19)

Oram and Davies (11) recently published their views on the indications and

contraindications for treating atrial fibrillation with direct current defibrillation Owing to the frequent electrocardiographic changes they do not consider atrial defibrillation advisable in connection with acute cardiac infarction In such cases a waiting period of three or preferably six months is recommended and even then careful consideration of the advantages and drawbacks of direct current defibrillation is necessary It should be born in mind, however, that in emergencies e g ventricular tachycardia or fibrillation, direct current countershock has hitherto proved safe even on patients with cardiac infarction (15 16)

Changes in the T wave and ST segment resembling those seen in hypokalemia were noted in 18 patients during the first few days after the attempted conversion These changes seemed to have no relationship to the treatment given before defibrillation

On follow up examination after 3 and 6 months the sinus rhythm was found to persist in 47 and 33 per cent respectively of the re examined patients

Summary

Non synchronized direct current countershocks were given for chronic atrial fibrillation to 36 patients on 43 occasions Normal sinus rhythm was reached on 38 occasions (88 per cent) SGOT was elevated in one case Conversion was most successful for young patients and for those with rheumatic heart disease Primary success seemed to be independent of the duration of atrial fibrillation total heart volume and left atrial size and of whether the position of the electrodes was transverse or oblique

Less advanced age and heart disease of rheumatic origin seemed to predict a good result In resistant cases countershocks given in one and the same session beyond a 4th or 5th shock proved to be useless If an attempt at defibrillation failed to produce sinus rhythm countershocks repeated later did not seem to improve on the result

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From the Institute of Work Physiology (Head K Lange Andersen M D) and the Institute of Occupational Health (Head J Glomme, M D) Oslo, Norway

Trainability of Old Men

By

Arne M Benestad

Physical training affects the functioning of the cardiovascular and respiratory systems of young people in a direction which makes them more fit for strenuous work (2) Old people, whose muscular performance is reduced by ageing processes are probably less capable of increasing their functional capacities by training, but very little information is available on this topic The present study of men aged 70—81 years was undertaken in order to elucidate this problem

Material

Thirteen subjects (in co-operation with Nasjonalforeningen for Folkehelsens Gerontologiske Institutt Oslo) volunteered to participate in the training experiments All of them were fit for physical activities and took regularly once a week part in gymnastic exercises arranged for older men In addition a few of them were also fond of outdoor recreational activities involving hiking in the woods during the summer season and skiing in winter time It is thus reason to infer that they were drawn from the physically most active part of the population Some of the subjects physical characteristics are given in table I

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A clinical examination which was undertaken prior to the training revealed degenerative disorders typical of old age (table II) Thus from a health point of view, these men did not distinguish themselves from the rest of the population Most of the subjects had the typical complaints associated with the disorders According to the investigator s judgement none of the disorders were so serious that they contradicted participation in the training programme

Training

The subjects trained regularly three times a week for a period of six weeks some for only five weeks The training took place in the laboratory under controlled thermal conditions and under constant medical supervision The men walked on a motordriven treadmill inclined 10 % After a preliminary warming up period for 5 minutes at a speed of 50 *m/min* the subjects performed a standard load at 70 m/min for another 5 minutes Those not being exhausted by the standard load walked then at a speed requiring about 50 % of their capacity for 2 minutes before they were exposed to a 5 minutes maximal load Maximal load was defined as a work rate engaging 60 % or more of the aerobic capacity Those of the subjects who were capable of it repeated this procedure once more during the training

TABLE I. Physical characteristics of the subjects (mean ± S.D.)

No. of subjects	Age (years)	Height (cm)	Weight (kg)	Sum of 10 skinfolds (mm)	Heart volume (ml)
13	75.5 ± 2.8	173 ± 5.7	70.7 ± 6.2	114 ± 32	800 ± 121

TABLE II. Degenerative disorders discovered by the clinical examination of 13 old men

	Cardio-vascular disorders					
No. of subjects	Abnormal ECG[1]	Hyper-tension[2]	Angina of effort	Cerebral arterio-sclerosis[3]	Disorders of locomotive apparatus	Signs of emphysema senilis[4]
13	9	2	1	2	2	7

[1] ECG changes at rest and/or during exercise include ectopic beats, rhythmic and conduction disorders, abnormal T wave and ST segment variations.
[2] One of these subjects had a hypertensive heart disease with cardiomegaly.
[3] Based on symptoms of vertigo or dementia.
[4] Based on clinical features.

session. That they were really working maximally was controlled by recordings of their heart rate and by spot sampling of the blood lactate after the exercise. The values of the latter ranged 27 mg—107 mg/100 ml blood thus indicating that the subjects were given a work intensity surmounting their ability to cover the energy demand by aerobic metabolism.

Methods

The clinical examination included ECG with 12 leads and X-ray of heart and chest in the erect position. The heart volume was determined according to Jonsell (7). The hemoglobin concentration, total amount of hemoglobin, sedimentation rate and serum cholesterol were examined, the blood taken in a post absorptive state. Blood volume was determined simultaneously by the dye dilution method, using Evans blue.

Maximal oxygen uptake and related respiratory and circulatory functions were measured by having the subjects bicycling on an ergometer of the mechanical braking type. Four submaximal work loads were performed, each lasting 5—9 minutes. The measurements were taken in the last minutes of the periods. At least two maximal work loads were performed, each lasting approximately 3 minutes, and the measurements taken in the last minute of the period. Only one maximal work load was performed in the same day.

Respiratory measurements were taken by using an open circuit system. Expired air was collected into a balanced 200 liter tank and samples of gas withdrawn for analyses by means of the 1/2 ml Scholander method (10). An Enghoff respiratory valve modified for heavy exercise was used (4).

Heart rates were recorded on an electrocardiograph Elema—Schonander 12 B during the last minute of the working period

Results

Oxygen cost of exercise at submaximal loads

The oxygen cost of exercise was not changed by the training (fig 1) The relationship between oxygen uptake and work output is clearly different from that of young subjects, in as much as oxygen uptake is higher at no load exercise and at light loads of work During severe stress however, the oxygen uptake be comes the same as in young subjects The figure demonstrates how well the level ling off of oxygen uptake at its maximal value was achieved

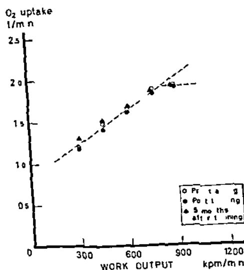


Fig 1 Average oxygen uptake in 13 old men during bicycling at different loads

Maximal oxygen uptake

The training period had no effect upon the aerobic capacity (table III) Not only was the average value the same, but also the range and variance was unaffected

Heart rate during exercise

Heart rate during walking on the tread mill at the standard load 70 m/min and inclination 10 per cent for 5 min utes, decreased gradually during the training period from an average of 131 to 117 beats/min (fig 2) This difference was statistically significant at the 1 % probability level If the oxygen cost of walking remained unaltered this would mean an increased oxygen pulse This concept was to some extent verified by the bicycle experiments undertaken be fore and after the training period These showed a tendency to lower heart rates at the same oxygen uptake level Five months after the training period the pre training relationship was reestablished as regards heart rate/oxygen uptake (fig 3)

TABLE III The effect of training upon maximal oxygen uptake heart rate and pulmonary venti lation (mean ± S D)

State of training	Max O_2 uptake (l/min)	(ml/min/kg)	Max O pulse	Highest recorded H R	Highest pulm vent (l/min BTPS)
Pre training	1 91±0 20	27±2 5	12 6±1 4	153±13	70±11
Post training	1 90±0 18	27±2 8	12 5±1 5	155±13	69±10
5 months after training	1 90±0 18	27±2 6	11 9±1 5	162±14	70±11

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Respiratory measurements were taken by using an open circuit system Expired air was collected into a balanced 200 liter tank and samples of gas withdrawn for analyses by means of the 1/2 ml Scholander method (10) An Lloyd-hoff respiratory valve modified for heavy exercise was used (4)

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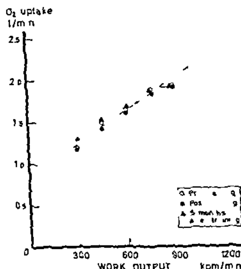


Fig 1 Average oxygen uptake in 13 old men during bicycling at different loads.

Maximal oxygen uptake

The training period had no effect upon the aerobic capacity (table III) Not only was the average value the same but also the range and variance was unaffected

Heart rate during exercise

Heart rate during walking on the tread mill at the standard load (0 m/min and inclination 10 per cent for 5 minutes decreased gradually during the training period from an average of 131 to 117 beats/min (fig 2) This difference was statistically significant at the 1 % probability level If the oxygen cost of walking remained unaltered this would mean an increased oxygen pulse This concept was to some extent verified by the bicycle experiments undertaken before and after the training period These showed a tendency to lower heart rates at the same oxygen uptake level Five months after the training period the pre training relationship was reestablished as regards heart rate/oxygen uptake (fig 3)

TABLE III The effect of training upon maximal oxygen uptake heart rate and pulmonary ventilation (mean ± S D)

State of training	Max. O_2 uptake l min	Max. O_2 uptake (ml min kg	Max. O pulse	Highest recorded H R	Highest pulm vent (l min BTPS)
Pre training	1 91 0 20	2 2 5	12 6 − 1 4	153 ± 13	70 ± 11
Post training	1 90 0 18	27 2 8	12 5 ± 1 5	155 ± 13	69 − 10
5 months after training	1 90 ± 0 18	27 2 6	11 9 − 1 5	162 ± 14	70 ± 11

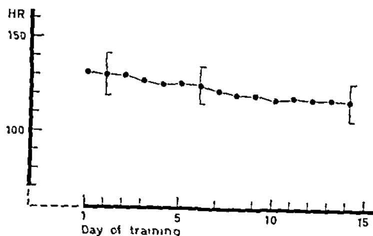


Fig 2 Effect of training upon mean heart rate in old men during steady state work performed on a treadmill at speed 70 m/min and inclination 10 % (The vertical lines denote ± SD)

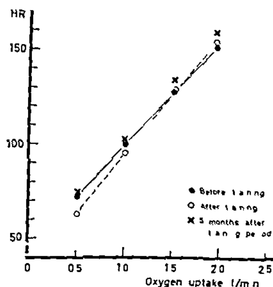


Fig 3 Heart rate/oxygen uptake relationship before and after training in old men

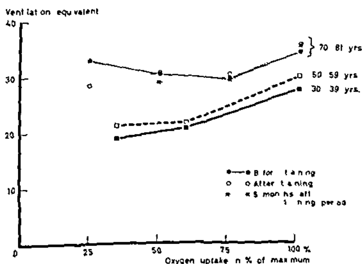


Fig 4 Ventilation/oxygen uptake relationship in old men compared with younger men at different steps of their oxygen uptake capacity

TABLE IV The effect of training upon the size of the heart blood volume hemoglobin and serum cholesterol (mean ± S D)

State of training	Heart volume (ml)	Blood volume (l)	Hemoglobin (g/100 ml)	Total hemoglobin (g)	Serum cholesterol (mg/100 ml)
Pre training	800±121	5 8±0 54	14 7±0 97	838±106	270±46
Post training	832±108	6 3±0 67	14 7±0 84	922±123	-
5 months after training	839±112	6 2±0 47	14 3±1 05	879±88	257±45

Pulmonary ventilation

No effect of training was seen on the pulmonary ventilation efficiency (fig 4) The lower efficiency with increasing age is clearly demonstrated in the figure The highest pulmonary ventilation was also unchanged by the training (table III)

The size of the heart

The size of the heart increased by the training from an average of 800 ml to one of 832 ml This difference was, however, not statistically significant at the 5 % probability level (table IV)

Blood volume and blood constituents

The blood volume increased from an average of 5 8 to 6 3 l (table IV) The difference is significant at the 5 % probability level but not at the 1 % level The same tendency to increase is noted for the total hemoglobin content and within the same limits of statistical significance Five months later the total hemoglobin amount had declined to the pre training value but the blood volume was unchanged

Discussion

A gradual decrease in the heart rate response to walking on the treadmill at the standard speed amounted to an average of 14 beats/min during the period of five weeks' training This indicated a training effect on the heart, and could also be a result of improved work efficiency Oxygen uptake was not measured during the walking, and thus no data are available to settle the latter problem However the heart rate in relation to oxygen uptake decreased as demonstrated in the bicycle experiments, but not to an extent explaining the reduction of walking pulse It is there fore good reason to suggest an improved work efficiency in walking The oxygen uptake in relation to work load on the bicycle remained unaltered Training in one exercise does thus not result in improved efficiency in other exercises

It should be noted that the oxygen cost of bicycling is greater in old men than in young men This is in contrast to Strandell s observation (11) He did not detect any effect of age upon work effi ciency during bicycling The lower work efficiency associated with old age is probably due to increased stiffness and reduced elasticity of the supporting joint tissue and also to some extent related to impaired co-ordination of the move- ments



Fig. 2. Effect of training upon mean heart rate in old men during steady state work performed on a treadmill at speed 70 m/min and inclination 10 %. (The vertical lines denote ± SD.)

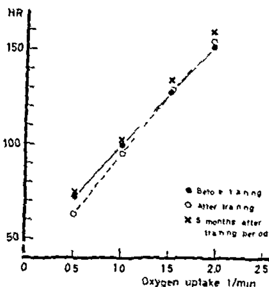


Fig. 3. Heart rate/oxygen uptake relationship before and after training in old men.

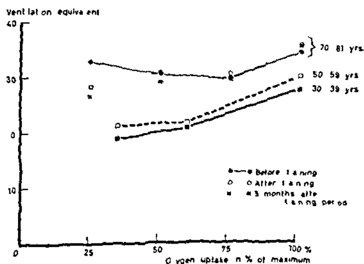


Fig. 4. Ventilation/oxygen uptake relationship in old men compared with younger men at different steps of their oxygen uptake capacity.

as it is also followed by reduced cardiac rate (3, 9)

No harmful effects of the training upon aspects of health were noted On the contrary, all subjects expressed spontaneously that the training was associated with increased general well being and a feeling of mental relaxation which they all appreciated

Summary

Thirteen old men, aged 70—81 years, volunteered in a training programme for 5—6 weeks The training resulted in a decrease of the heart rate oxygen uptake relationship This indicates an improved work efficiency There was also noted a significant rise in blood volume and total amount of hemoglobin, and a tendency to enlargement of cardiac volume These findings are assumed to be beneficial reactions They are together with the reduced heart rate, interpreted as the cause of a greater oxygen pulse and a possibly greater stroke volume Thus the final result is an improved pumping capacity of the heart The training did not affect the aerobic work capacity The influence on the mental well being however was a marked re sponse to the training

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The maximal oxygen uptake remained unaffected during the training regimen This does not necessarily mean that the trainability of old men is completely lost The duration and intensity of the training was as much as the medical supervisor wanted to expose these old men to for a period of five weeks It does not mean, however, neither that the training represented the upper limit for muscular activity at this age, nor that they could not tolerate a greater training load and training intensity It is therefore possible that a harder training would have affected the capacity of the aerobic muscle metabolism Experience from training of young men has shown that the oxygen uptake capacity is only little affected by 5—6 weeks of hard muscular work Andersen et al (2) trained a group of young students for 5 weeks and noted an increase in maximal oxygen uptake of only 6 % Also subjects with large differences in habitual physical activity may have the same capacity of the aerobic muscle metabolism (1) The practical implication of this result is that one cannot expect to increase the aerobic capacity of healthy old men by under taking physical training

The five weeks of training had a noticeable effect upon the functioning of the heart and in the same direction as seen in younger age groups The heart rate oxygen uptake relationship decreased significantly, and a tendency to enlargement of the cardiac volume was found This indicates an increased stroke volume The definite evidence for this conclusion is not at hand, because cardiac output was not measured Reduced rate and increased stroke volume is generally considered to be a beneficial reaction, increasing the pumping capacity of the heart

The maximal heart rate did not change with training in these old men This is in contrast to observations in young men, in whom maximal heart rate decreases with training (2)

The pulmonary ventilation efficiency was unaffected by the training Based on the findings of differences between superior athletes and normal people it has been postulated that training improves the ventilation efficiency The ventilation efficiency of these old men was clearly inferior to that found in young men The pulmonary ventilation required to take up 1 l oxygen was 30 l in the old men, compared with 25 l in young men (5)

The blood volume increased by 8 % during the training regimen, which is a statistically significant figure Blood volume is known to be affected by training in young men, and may be the primary training effect The higher blood volume is associated with increased amount of total hemoglobin (6, 8) This evidence can only mean that the training process stimulates increased hemoglobin formation The mechanism for this is not clear The increased blood volume and total hemoglobin must be looked upon as a beneficial reaction It should be noted that 5 months after the training period the amount of total hemoglobin had returned to pre-exercise values, while the blood volume and heart volume were the same It might well be that the enlargement of the heart volume is related to increased blood volume, but this is probably not a simple relationship

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From the Institute of Pathology and Anatomy (Head Osmo Jarvi, M D), Turku University, Turku and from Mikkeli Provincial Hospital (Head Jaakko S Aalto, M D), Mikkeli, Finland

Metastases of Malignant Melanoma in the Stomach and Small Intestine

By

HEIKKI BÄCKMAN and LEO DAVIDSSON

Haematogenic metastases produced by malignant tumours are generally rare in the stomach wall, about one quarter have been found to be connected with malignant melanoma As a rule secondary tumours in the intestinal tract are most frequent in the small intestine Walther (13) in his thorough pathologico-anatomical study of 3 584 cases of carcinoma, found an incidence of 1 14 per cent for metastases in the small intestine Of these too a quarter were connected with malignant melanoma the primary tumour was on the skin in 9 cases and in the eye in one case

Malignant melanoma in the stomach and small intestine is almost without exception a metastatic tumour It has only *very rarely been noted as a primary* growth in the wall of the oesophagus, stomach or small intestine (2, 10, 12) Banzet et al (1) described 70 cases of melanoblastoma collected from the literature in which metastases were noted in either the stomach or the small intestine or both According to Reed et al (10) considerably fewer than 100 cases of malignant melanoma in the wall of the stomach or the small intestine had been reported in the literature up to 1962 There are, therefore few studies of metastatic tumours in the stomach and the small intestine and for this reason it was considered that a report might usefully be made on the following case diagnosed from a biopsy specimen

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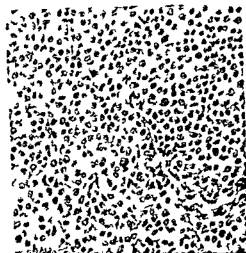
Case report

The case was that of a 58-year-old widow who had suffered from hypertension for several years On Jan 7 1959 she had had a birth mark removed from the skin of her right mammary gland, together with ulceration that had developed one year earlier

Histological examination (Prof Osmo Jarvi) of the specimen taken from the skin of the breast revealed a flattened spongy swelling 1 5 cm in diameter and 0 5 cm high covered by thinned mostly regular epidermis In one place however the surface of the swelling was ulcerated (fig 1) Over the whole area of the swelling and over an area of approximately the same size in the corium proper, extend



F g 3 Detailed ew of the fusiform ell zone. Haematox l n-eos n s a n ng × 75



F g 4 Detailed ew of the round cell a ea No p gment could be demonstra ed n his area by s lver stain ng Haema oxylin-eosin staining × 240

spec mens taken on this occas on from the skin of the breast and from the armp

In Nov 1960 the pa ent onsul ed the Med cal Ou patient Depa tment of Hels nki Un ersi y Central Hosp tal She complained of fa gue loss of weight and an almost con nuous ach ng pain in the ep gastrium whi h unconnec ed w th meals had gone on for o er a mon h No om ting had occur ed Rad og aph revealed susp c ous-looking or gan c changes in the pos erior wall of the s oma h h le cholecys og aphy ga e a n ga e resul Gas roscopy showed an u a on n the lesser curva u e surrounded b a wall l ke fold of une en appearance In e g a r curva ure the e we e *two* pseu lopolyps, one of hem w h an ulce a ed su fa e The n erp eta on of he f nd ngs was l a he e were wo eviden ly ben gn u nou s n the g ea er curva ure and an ul e a on wh h seemed malignan n the lesser curvature Laboratory tests sho ed slight anaem a Hb 10 0 g %, erythrocytes 3 68 m ll /mm³ MCH 27 leucocytes 7 300 ESR 30 mm 1 h GOT 16 un ts CRP + + No albumin sugar or bile p gments were found n the urine For blood in the faeces the benz dine test was pos e and the guaiac test negati e paras te ova D phyllobothrium latum were found n the faeces

The patient was aga n admitted to Mikkeli Pro incial Hosp tal on Nov 14 1960 on the recommenda on of the Med cal Outpatient Department of Hels nki Un vers ty Cent al Hosp tal which ad ised surgery under the d agnos s of gas ric tumour The patient s general cond on was rela ely good though pyrex a ad 37 6 C persisted and the patient s stools had been dark for over a month. Tenderness to palpat on but no resistance was noted n the ep gastrium and the left iliac fossa Radiography of the lungs and the s nuses ga e normal find ngs ESR was 27 *mm l h and hypoch om c* anaemia was p esent For the faeces he benz d ne test alone was pos e but aga n no albumin or sugar was found n the urine Laparo omy was performed on Nov 24 1960 and some asc es was no ed n the abdominal ca ity

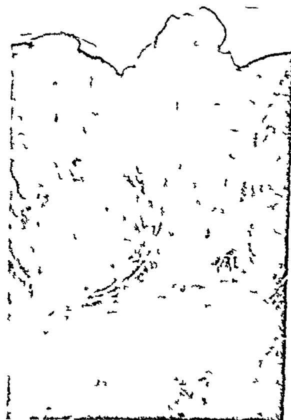


Fig 1 General view of the primary tumour removed from the skin of the breast A superficial zone of predominantly fusiform cells and a deeper zone of round cells can be discerned Haematoxylin-eosin staining × 15



Fig 2 Pigment in the superficial layer of the primary tumour Masson s silver staining × 45

ing to some depth into the subcutis there was tumour tissue composed of definitely irregular naevus cells The cells occurred partly in the form of delimited islands partly as extensive continuous streaks Atypical cells were also visible inside the epidermis Some of the cells were fusiform others fairly round or angular The nuclei varied considerably in size showing a coarse chromatin network and large nucleoli while mitoses were very frequent The superficial swelling was moderately infiltrated by inflammatory cells these were primarily lymphoid and plasma cells though polymorphonuclear leucocytes were also visible Melanin pigment occurred in a fairly limited section of the swelling area and the naevus cells in this zone were mostly fusiform (fig 2) In the tumour focus of the corium the cells or almost all of them seemed to be round to contain little plasma and to have nuclei of only small variation in size whereas mitoses were numerous This part of the tumour seemed to be completely free from pigment a finding corroborated by melanin pigment staining with Fontana s silver nitrate solution In depth the tumour extended to the margin of the specimen the tumour had thus evidently not been removed in its entirety Laterally the tumour tissue extended at the stylus in the epidermis proper somewhat beyond the swelling Details of the zone of fusiform cells and of the round cell area are shown in figs 3 and 4

Less than three weeks after the removal of the primary tumour the patient was admitted to Mikkeli Provincial Hospital where ablation of the mammary gland and evacuation of the right axillary fossa were performed on Jan 28 1959 No tumour tissue was noted in the

and anaemia, the symptoms displayed in the present case, in general suggest malignancy This particular patient had in addition persistent epigastric pain, attributable to the econdary foci in the stomach The tenderness of the left iliac fossa may similarly be explained by the metastases found in the area of the small intestine A tumour in the small intestine may lead to invagination a phenome non not uncommon in conjunction with the metastases of malignant melanoma in the small intestine (18) In the present case no symptoms of obstruction were noted, although the biggest metastasis found in the jejunum was approximately the size of an egg Intestinal metastases do not usually become big enough to be palpable They have been found to vary in diameter only from some 2 5 to 3 0 cm (13) and the ulcerations observed have been flat In the present case the ulceration noted in the lesser curvature of the stomach was surrounded by a wall like fold of uneven appearance and on gastroscopy the ulceration seemed malignant No diarrhoea was noted but medical history revealed bleeding from the bowels though it had ceased before admission to hospital No peritoneal irritation suggestive of perforation was noted this has not generally been one of the findings in reports on secondary intestinal tumours (14)

Intestinal metastases of malignant mel anoma have seldom been diagnosed in vivo 11) This is partly because of the rarity of intestinal metastases in connec tion with tumours with a tendency to metastasize because of their smallness the metastases are not easy to discover by radiography In the present ca e radiography revealed suspicious looking or ganic changes in the stomach, but the small intestine was not radiographed Few neoplasms give a similar radiographic picture Apart from malignant melanoma, argentaffinoma, among oth ers, has been reported as producing multiple tumours in the tract of the small intestine (3) Radiography can therefore suggest malignant melanoma if several separate tumours of different sizes are noted in the mucosa of the stomach or of the small intestine, in the case described by Beirne (3) malignant melanoma was suspected on the basis of the multiple polyps seen in the small intestine on radiography Similarly, gas tric metastases of malignant melanoma were suspected on the basis of the clinical picture and gastric radiography in the cases reported on by Julitz (7) and Grigorian (5), in these cases the diagnosis was verified histologically When the metastases are in the stomach the diagno sis can be verified from gastroscopy by exfoliative cytological examination as was done in the case described by Reed et al (10)

Morphologically the primary tumour and the metastasis are usually similar In the later stages the structure characteristic of the tumour may disappear and the metastases then developing may be less and less differentiated In the present case the tumour tissue of the metastases was fairly solid though its demarcation from its environment was somewhat vague On staining the cells were com patible with melanoma but the charac teristic pigment of malignant melanoma could not be demonstrated with cer tainty Walther (13) has found amelanot



Fig 5 Metastasis in the submucosa of the small intestine extending in part into the muscularis propria van Gieson staining × 10

Inside the stomach in its anterior wall a knotty tumour could be palpated and a biopsy specimen of the tumour was taken by gastrostomy In the small intestine over a length of 60 cm six separate tumours were noted inside the lumen and obstructing it The biggest was situated some 50 cm from the gastric end of the small intestine it was the size of an egg and bluish in colour Thick infiltration continued from it all the way to the mesenteric root No signs of occlusion were noticeable The case was considered inoperable and only the biopsy specimen was taken

Histological examination (Prof Osmo Jarvi) of the specimen taken from the stomach revealed somewhat thinned mucosa in structure primarily that of the gastric body which coated the tumour swellings in the submucosa In general no tumour tissue was observable in the mucosa though at one spot the neoplastic tissue obviously penetrated through the muscularis mucosa and the mucous coat Here the tumour tissue was partly necrotic The mucosa was to some extent oedematous and exudate cells in it were slightly increased The glands were shorter than usual and in addition to glands of gastric body type there were mucus tubes corresponding to so-called pseudopyloric structure The tumour tissue was in form of a fairly continuous focus diameter over 1 cm, in the submucosa The cells were mostly round and often highly reminiscent of the tumour cells which especially in the specimen described above occurred in the corium proper There was however, more variation in the cells. Melanin pigment could not be demonstrated even by Fontana s silver method

The specimen taken from the small intestine revealed a similar tumour focus some 7 mm in diameter, in the submucosa from whence it spread by infiltration into the inner layer of muscularis propria (fig 5) The mucosa of the small intestine on the surface of the tumour was fully regular Here, too the tumour was made up of extensive continuous islands and streaks and its cell structure was identical with the part of tumour seen in the corium proper of the original naevus The cells were fairly small and varied little in size Melanin could again not be demonstrated in this tumour focus with Fontana s silver solution The gastric and intestinal tumour foci examined were obviously metastases of the skin melanoma which had been removed earlier The tumour had furthermore evidently produced unpigmented metastases

The patient was discharged from hospital on Dec 5 1960 Her general condition was then unchanged but at the follow up examination of Dec 27 1960 it was found to have deteriorated haemoglobin value was 8 5 g % and ascites was present The patient died on Feb 10 1961 No autopsy was performed

Discussion

Symptoms evoked by secondary tumours of the small intestine may be very indefinite Lack of appetite loss of weight

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ic metastases in connection with malignant melanoma in about half the number of cases with metastases They have usually occurred in the lungs, the liver and the thyroid gland, and even, though rarely, in the heart muscle (9), while only the metastases in the brain were almost all pigmented According to Farrell (4) unpigmented melanomas are less differentiated than pigmented, and are therefore the more malignant of these two types It is not known whether there were metastases elsewhere in the organism of the present patient When the metastases in the gastric and intestinal tract were studied the tumour tissue was found to be solid and unpigmented, and the course of the disease progressed rapidly

In those cases in which the malignant melanoma grows locally, directing itself systematically towards the closest lymph glands, removal of the tumour in a sufficiently early phase may produce a satisfactory result James (6) reports a recovery in 32 9 per cent of such cases after an observation period of 5 years from the removal of the primary tumour and in 19 per cent after a period of 10 years On the other hand it is known that metastases of malignant melanoma may manifest themselves at a very late stage, up to 15 years after the removal of the primary tumour (8), and the observation period must therefore be sufficiently long when prognosis is assessed In the present case the general symptoms produced by the metastases of malignant melanoma developed over the 18 months immediately following the removal of the primary tumour The appearance of metastases was to be expected, since tumour cells were found to extend beyond the excision limit of the tumour in connection with the removal of the pigmented naevus Ulceration of the naevus had, however, been present for about a year before its excision, and radical removal of the tumour was therefore performed at too late a stage

Summary

Tumours caused by malignant melanoma in the alimentary tract have, with rare exceptions, been metastases Their clinical diagnosis is difficult, since the symptoms they produce may be very indefinite, and since these metastases are very rare The disease may, however, be suspected on the basis of the patient's medical history and the radiographic finding, though the diagnosis has usually not been definite before a histological study has been made

In the present case metastases of malignant melanoma in the stomach and the small intestine were diagnosed on laparotomy The general symptoms of the disease had set in about 18 months after the excision of pigmented naevus from the skin of the breast The development of metastases was to be expected since after the removal of the naevus the tumour cells were found to have extended beyond the excision limit When the prognosis in these cases is assessed the observation period must be made sufficiently long since secondary foci have been found to appear as much as 15 years after the removal of the primary growth

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From King Gustaf V Research Institute (Head G Birke, M D), the Department of Surgery (Head J Adams Ray, M D) and Internal Medicine (Head H Lagerlöf, M D), Karolinska Hospital Stockholm Sweden

Lipid Metabolism and Trauma

III Plasma Lipids and Lipoproteins in Burns

By

GUNNAR BIRKE, LARS A CARLSON and STEN OTTO LILJEDAHL

Trauma is becoming an increasingly important condition from the clinical point of view This is due not only to the increased number of accidents but also to the present day awareness that many acute diseases expose the body to trauma in the acute phase The treatment of trauma requires an extensive knowledge of the metabolic change it produces, and it is known that among these changes are effects on lipid metabolism Thus the concentration of the free fatty acids (FFA) of plasma is increased (25 9) and the cholesterol level decreased (4 24) In dogs there is also an increase in the content of triglycerides in the liver 24 hours after trauma (9) The changes in plasma lipids in man after trauma have now been studied in greater detail, in relation to the degree of trauma and to their course during different phases of the recovery period Burns were choosen as a model for trauma due to their easy classification and to the knowledge we have previously accumulated on metabolic changes after this form of injury

Submitted for publication March 19 1965

Material

Most of the cases studied were admitted to the hospital within 3 hours of the burn and none later than 6 hours

Cases were divided into three groups according to the degree of severity of the burn

Group 1 = small burns the value being < 15/5 % for the extent of burn expressed as *total extent of burn/extent of third degree of burn*

Group 2 = medium sized burns 15—30/5—15 %

Group 3 = extensive burns > 30/15 %

In the analysis of mortality risk Bull & Fisher s type of analysis was used (6) Among the 38 patients studied 10 were women The composition of the material with regard to age extent and degree of burn and calculated risk of mortality is given in table I There were no deaths in groups 1 and 2 In group 3 six of the studied cases died one (90/90) on day 5 (circulatory shock) one (85/85) on day 8 (septecemia) one (55/50) on day 10 (pulmonary burn) one (50/50) on day 16 (pulmonary burn) one (40/55) on day 24 (gastrointestinal bleeding) and one (45/49) on day 30 (pulmonary emboli) The calculated mortality risk was 1 0 for all these deaths except the last mentioned case where the risk was 0 9

The patients in group 2 and 3 were for the first 24 hours after the burn given colloids and

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TABLE I The composition of the material with regard to age, extent and degree of burn and calculated risk of mortality

Group	No of cases		Age (years)	Burn of body surface (%) Total surface	3rd degree	Mortality risk[1]
1	7	Mean	36	10	4	0.1
		Range	14—55	7—15	2—5	0—0.2
2	14	Mean	47	20	7	0.3
		Range	24—71	15—30	2—10	0.1—0.7
3	17	Mean	39	46	35	0.7
		Range	10—68	30—85	15—85	0.1—1.0

[1] Calculated according to Bull and Fischer (1954)

electrolytes, 1.5 ml and 1.0 ml respectively per kg body weight and per each one per cent of body surface burned Plasma blood and 20 per cent human serum albumin were the only colloids used Plasma and blood were given in the proportion 4 : 1 On the second and third days, at least half this amount was generally given In addition one liter of glucose was given intravenously on each of these days

On the subsequent days blood and fluid were given on the basis of the result of the fluid balance chart Oral fluid therapy was avoided for the first 5 days in group 2 and for the first week in group 3

Three grams of human gammaglobulin were given daily from the third day and thereafter for 5—6 days Data on the parenteral treatment of groups 2 and 3 are recorded in table *II*

All the patients were initially treated by the exposure method The excisions of the third degree burns were not started until the 14th day Skin grafting was not started until a few days after the [illegible]ion, and the grafts were then applied dire[illegible] on the excised surface without secondary dressings In most of the cases the third degree burns were grafted within 6 weeks

Methods

Heparin was not given to the patients during this study Blood samples were withdrawn daily from a catheter inserted into the brachial artery during the first week The blood was then taken by venepuncture On the day of the burn the blood samples were taken immediately after admission to the hospital On all other days the blood was taken in the fasting state in the mornings During the period of parenteral nutrition at least 4 hours elapsed between the end of the preceding infusion and the blood sampling Blood was taken the day before and not earlier than 3—4 days after the skin grafting Every patient who survived was studied after being discharged from hospital about 6—12 months after the burn

The blood samples were collected in heparinized tubes and either centrifuged immediately and the plasma extracted or stored for some hours at + 4° C The plasma FFA was determined according to Dole (15) and other plasma lipids as described previously from this laboratory (7, 8) The plasma lipoproteins were separated by centrifugation in the Spinco Model L preparative ultracentrifuge according to Bragdon et al (5) and the lipoproteins were recovered and analyzed

TABLE II Average amounts of fluid protein and calories given intravenously to groups 2 and 3 and average urine volume during the first week after trauma. Group 3 received only intravenous nutrition during this period while most patients in group 2 in addition were able to drink during days 5 to 7

Group	Period[1] (day)	Fluid (l)	Protein (g)	Calories (cal)	Urine vol (l)
2	0–1	5.6	66	1 400	1.2
	1–2	4.8	62	1 300	1.4
	2–3	3.2	45	1 300	1.7
	3–4	3.0	43	1 200	1.5
	4–5	2.8	40	1 200	1.6
	5–6	2.4	45	1 100	1.4
	6–7	2.4	44	1 100	1.7
3	0–1	8.5	135	1 800	1.3
	1–2	6.3	92	1 600	1.4
	2–3	5.3	76	1 500	1.5
	3–4	4.0	74	1 400	1.9
	4–5	4.0	59	1 400	1.7
	5–6	4.0	55	1 200	1.9
	6–7	3.3	57	1 100	1.8

[1] Day 0 is the day of the injury

for lipids as described earlier (10) The lipoproteins floating to the top at densities 1 006 and 1 063 are called *very low* and *low density* lipoproteins respectively The remaining lipoprotein fraction is called the *high density* lipoproteins

Total serum protein concentration was determined in duplicate by Kjeldahl's method the mean being taken

Paper-electrophoresis of the serum proteins was carried out in veronal buffer (pH 9 6 ionic strength 0 1) with 5 mA per strip

The statistical treatment was done according to Snedecor (23)

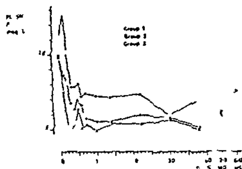


Fig 1 Mean concentration of FFA in plasma during the course of small (group 1) medium (group 2) and extensive (group 3) burns During the first week blood was taken from an arterial catheter and thereafter from an antecubital vein

Results

Plasma lipids

The values for the plasma lipids in groups 1, 2 and 3 are given in tables III, IV and V, respectively

The *concentration of FFA* in the three groups is shown in fig 1 There was initially an elevated level of FFA in the plasma of all groups The magnitude and the duration of this increase was correlated to the extent of burn Already on day 1 the concentration of FFA was significantly lower in group 1 than in group 3 ($P < 0.05$) About ten days after the trauma the FFA level in group 3 was significantly higher than in both groups 1 and 2 ($P < 0.05$)

The *concentrations of cholesterol and phospholipids* followed each other as seen in figs 2 and 3 These lipids decreased progressively during the first 7 to 10 days The most pronounced decrease was for cholesterol in group 3 at day 10 when an average level of 125 mg/100 ml plasma was reached After reaching a minimum level around day 7 to 10 these

TABLE III The concentrations of cholesterol, phospholipids, triglycerides and FFA in fasting[1] plasma

Day[1]		0	1	2	3	4
Cholesterol (mg/100 ml)	Mean	201	186	180	174	164
	S E of mean	16	21	12	14	8
	No	4	6	6	7	6
Phospholipids (mg/100 ml)	Mean	239	209	208	215	206
	S E of mean	20	19	18	14	15
	No	4	6	6	7	6
Triglycerides (mmoles/l)	Mean	1 11	1 05	1 14	1 27	1 13
	S E of mean	0 11	0 19	0 10	0 23	0 18
	No	4	6	6	7	6
FFA (mEq/l)	Mean	0 99	0 72	0 51	0 48	0 54
	S E of mean	0 19	0 12	0 08	0 11	0 09
	No	4	6	6	7	6

[1] = On day 0, which is the day of the injury, blood was drawn immediately after admission of the

TABLE IV The concentrations of cholesterol phospholipids, triglycerides and FFA in fasting[1] plasma

Day[1]		0	1	2	3	4	5
Cholesterol (mg/100 ml)	Mean	266	227	211	193	172	160
	S E of mean	30	17	13	13	11	10
	No	5	13	13	12	12	11
Phospholipids (mg/100 ml)	Mean	314	261	239	236	215	211
	S E of mean	21	17	12	15	15	12
	No	5	13	13	13	12	12
Triglycerides (mmoles/l)	Mean	1 55	1 22	1 27	1 35	1 24	1 27
	S E of mean	0 28	0 17	0 12	0 18	0 13	0 09
	No	5	13	13	13	12	12
FFA (mEq/l)	Mean	0 98	0 86	0 81	0 67	0 63	0 69
	S E of mean	0 18	0 10	0 09	0 03	0 06	0 07
	No	4	13	13	13	11	12

[1] = On day 0 which is the day of the injury, blood was drawn immediately after admission of the

lipids slowly increased The initial level of cholesterol was not reached until 6 to 12 months after the trauma in groups 2 and 3 There was no significant difference in the cholesterol levels of the groups at day 0 However, at day 10 the cholesterol level in group 3 was significantly lower than in group 1 ($P < 0.01$) and group 2

in group 1

5	6	7	8–12	13–17	18–25	26–33	34–41	1–3 months
165	162	154	165	177	185	190	232	240
9	18	12	8	13	10	28	15	30
7	4	3	6	6	5	3	2	5
199	184	207	194	190	215	216	240	255
13	10	15	10	13	12	25	30	28
7	4	3	6	6	4	3	2	5
1 31	0 92	1 14	1 27	1 16	1 56	0 05	1 55	1 39
0 32	0 14	0 36	0 25	0 16	0.24	0 23	0 20	0 27
7	4	3	6	6	5	3	2	5
0 63	0 51	0 53	0 49	0 54	0 53	0 56	0 49	0 58
0 12	0 09	0 17	0 06	0 05	0 08	0 10	0 09	0 04
6	4	2	6	6	6	3	2	5

patients

in group 2

6	7	8 12	13–17	18–25	26–33	34–41	1–3 months	6–12 months
151	149	168	177	182	178	218	219	257
11	8	7	6	9	13	13	16	16
11	12	14	13	14	10	8	9	13
201	191	211	199	218	215	229	267	303
13	9	11	7	11	15	12	16	19
11	12	14	13	14	10	7	9	13
1 70	1 27	1 47	1 31	1 39	1 46	1 51	1 52	1 32
0 07	0 12	0 19	0 09	0 11	0 20	0 22	0 27	0 19
11	12	14	13	14	10	7	9	13
0 67	0 58	0 55	0 55	0 59	0 57	0 51	0 61	0 90
0 10	0 06	0 04	0 04	0 05	0 05	0 05	0 04	0 06
11	12	14	12	14	10	6	8	13

patients

P 0 001) Even 30 days after the trauma the concentration of cholesterol was lower in group 3 than in group 2 (P < 0 05) while no differences were observed between these groups after 6 to 12 months

The concentration of triglycerides showed no consistent changes in group 1 and 2

TABLE V The concentrations of cholesterol, phospholipids, triglycerides and FFA in fasting[1] plasma

Day[1]		0	1	2	3	4	5
Cholesterol (mg/100 ml)	Mean	243	224	188	174	155	138
	S E of mean	25	19	11	10	8	6
	No	13	14	16	16	17	17
Phospholipids (mg/100 ml)	Mean	299	266	244	245	231	212
	S E of mean	33	21	11	11	11	11
	No	13	14	16	16	17	17
Triglycerides (mmoles/l)	Mean	2 03	1 77	1 76	1 77	1 69	1 62
	S E of mean	0 39	0 34	0 23	0 24	0 20	0 25
	No	12	14	16	16	17	17
FFA (mEq/l)	Mean	1 12	1 28	1 10	0 78	0 78	0 80
	S E of mean	0 16	0 21	0 19	0 06	0 07	0 08
	No	13	12	15	16	16	15

[1] = On day 0, which is the day of the injury, blood was drawn immediately after admission of the

TABLE VI The concentrations of cholesterol, phospholipids and triglycerides in the high density low
Mean ± S E of mean (no of patients)

	High density				Low density
Day	Ch	Ph	Tg	R	Ch
0–1	60±7 (5)	132±12 (5)	0 19±0 05 (5)	0 45±0 03 (5)	169±26 (5)
3	46±6 (6)	114±10 (6)	0 25±0 04 (6)	0 40±0 02 (6)	112±8 (6)
5	38±4 (6)	98±7 (6)	0 20±0 04 (6)	0 39±0 04 (6)	90±5 (6)
7	29±4 (6)	87±8 (6)	0 21±0 05 (6)	0 03±0 02 (6)	78±4 (6)
9–11	27±3 (6)	79±7 (6)	0 20±0 05 (6)	0 34±0 04 (6)	73±8 (6)
19–22	41±6 (5)	97±6 (5)	0 20±0 04 (5)	0 42±0 06 (5)	95±11 (5)
26–29	56±5 (5)	117±11 (5)	0 30±0 08 (5)	0 49±0 04 (5)	91±19 (5)

Ch = cholesterol (mg/100 ml) Ph = phospholipids (mg/100 ml) Tg = triglycerides (mmoles l)

in group 3

6	7	8—12	13—17	18—25	26—33	34—41	1—3 months	6—12 months
1 0	129	125	140	150	152	187	198	244
5	8	7	6	6	11	15	20	16
15	12	16	13	12	11	8	8	10
193	191	187	187	209	209	224	233	262
9	9	6	10	9	16	14	19	14
15	12	16	13	12	11	8	8	10
1 69	1 54	1 54	1 40	1 43	1 75	1 38	1 80	1 36
0 25	0 16	0 14	0 13	0 15	0 34	0 22	0 12	0 16
15	12	16	13	12	11	8	8	10
0 72	0 74	0 72	0 71	0 73	0 59	0 69	0 61	0 75
0 05	0 08	0 05	0 05	0 06	0 06	0 06	0 05	0 05
15	11	16	12	12	11	8	7	10

pat ents.

dens ty and very low density lipoprotein classes. Six patients belonging to group 3 were studied

			Very low density		
Ph	Tg	R	Ch	Ph	Tg
1 6 23 5	0 50–0 08 (5)	1 36±0 05 (5)	16±6 (4)	19±6 (4)	0 42±0 17 (4)
91 6 6	0 63+0 06 (6)	1 23–0 03 (6)	16±3 (5)	23±2 (5)	0 57+0 09 (5)
81 11 6	0 76 0 19 (6)	1 16±0 07 (6)	7+5 (5)	12±6 (5)	0 24–0 13
9 5 6	0 90+0 11 6	1 00±0 04 (6)	12+5 (5	21±6 (5)	0 48±0 18 (5)
1 6 6	0 73_0 14 (6	1 03±0 08 (6)	9 4 (5	17±7 (5)	0 36±0 18 (5
8 5 5	0 63 0 09 ()	1 14–0 09 5	6 1 (4	10±1 (4)	0 37±0 08 (4)
6 8 5	0 61 0 12 5	1 16±0 13 (5)	24–16 4	39±22 (4	1 14±0 89 4)

R ratio cholesterol phospholipids.

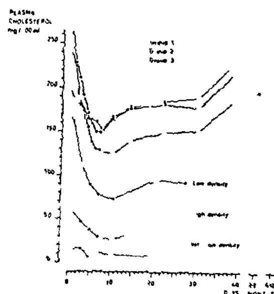


Fig 2 Mean concentration of cholesterol in plasma during the course of small (group 1) medium (group 2) and extensive (group 3) burns The average concentrations of cholesterol in three different plasma lipoprotein fractions the very low the low and the high density lipoproteins from 6 patients in group 3 are also given

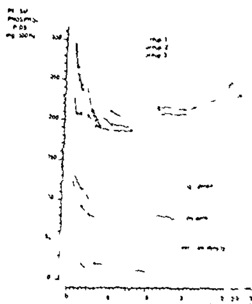


Fig 3 Mean concentration of phospholipids in plasma during the course of small (group 1) medium (group 2) and extensive (group 3) burns The average concentrations of phospholipids in three different plasma lipoprotein fractions the very low, the low and the high density lipoproteins from 6 patients in group 3 are also given

In group 3 there was a tendency towards a decrease from an initially high level (fig 3) There was, however, no statistically significant difference between the triglyceride levels on day 0 and after 6 to 12 months in group 3 On the other hand, the concentration of triglycerides was statistically higher ($P < 0.05$) in group 3

TABLE VII The concentrations of proteins and albumin in plasma in groups 2 and 3

Group	Day[1]		0	1	2	3
2	Plasma proteins	Mean	6 8	6 5	5 9	5 9
	(g/100 ml)	S E of mean	0 14	0 33	0 32	0 34
		No	11	6	5	7
	Albumin	Mean	4 35	3 76	3 35	3 29
	(g/100 ml)	S E of mean	0 17	0 25	0 35	0 28
		No	11	6	5	7
3	Plasma proteins	Mean	6 7	6 3	5 8	5 9
	(g/100 ml)	S E of mean	0 30	0 23	0 17	0 14
		No	11	15	16	16
	Albumin	Mean	4 39	3 88	3 41	3 15
	(g/100 ml)	S E of mean	0 21	0 19	0 13	0 13
		No	11	15	16	16

1 — On day 0 which is the day of the injury blood was drawn immediately after admission of the

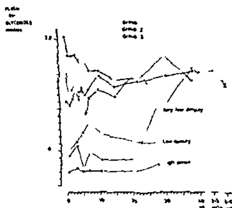


Fig 4 Mean concentration of triglycerides in plasma during the course of small (group 1), medium (group 2) and extensive (group 3) burns *The average concentrations of triglycerides in three different plasma lipoprotein fractions the very low the low and the high density lipoproteins from 6 patients in group 3 are also given*

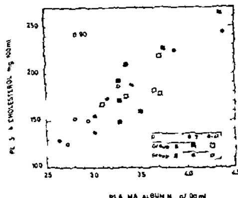


Fig 5 The relationship between the mean concentrations of cholesterol and of albumin in plasma on various days *during the course of medium (group 2) and extensive (group 3) burns* For a given day the mean concentration of cholesterol was plotted against the mean concentration of albumin

than in group 1 on day 0 When calculated on the logarithms for the triglycerides which eliminates their skewness of distribution (7) this difference was significant at the 1 per cent level All other calculations of the triglycerides were also done on the logarithms These calculations showed the same results as those performed on the unconverted values

4	5	7	8−12	13−17	18 25	26−33	34−41
6 3	6 7	6 4	6 8	7 0	7 3	7 4	7 4
0 26	0 52	0 28	0 28	0 31	0 39	0 45	0 58
9	4	7	8	6	7	6	3
3 28	3 50	3 28	3 10	3 13	3 66	3 72	3 72
0 73	0 40	0 29	0 21	0 27	0 35	0 51	0 50
9	4	7	8	6	7	6	3
8	5 8	6 0	6 2	6 6	6 8	6 8	6 7
0 76	0 15	0 23	0 23	0 18	0 32	0 32	0 25
15	17	14	11	13	11	4	5
3 07	3 07	2 63	2 73	2 88	2 95	2 81	3 27
0 18	0 13	0 13	0 18	0 13	0 19	0 29	0 30
15	17	14	11	13	11	4	5

Patients

Plasma lipoproteins

The lipid composition of the three main classes of plasma lipoproteins in group 3 is given in table VI and is also shown in figs 2, 3 and 4 The most consistent and pronounced changes were those in the *concentrations of cholesterol and phospholipids* in the high and low density lipoproteins These lipids decreased progressively in both classes, reaching a minimum level on day 10 On day 26—29 the lipid content of the high density class was almost completely restored However, at that time the cholesterol and phospholipid content of the low density lipoproteins was only about 55 per cent of the initial value The *cholesterol/phospholipid ratio* in both the high and the low density class decreased significantly to a lowest value on day 7—11, indicating a change in the composition of both these lipoprotein classes

Plasma proteins

The concentrations of the total proteins and of albumin in plasma in groups 2 and 3 are given in table VII The albumin concentration decreased progressively in both groups, the decrease being most pronounced on day 7—12 The decrease was most pronounced in group 3 The albumin concentration was still decreased 6 weeks after the burn

Fig 4 shows that there is for both groups 2 and 3 a close correlation between the concentration of albumin and of cholesterol in plasma In respect of this correlation the period of decrease resembled the period of increase of albumin and cholesterol, as is evident from the figure

Discussion

Trauma is an important stimulus to metabolic changes, and the degree of these has been found to be well correlated with the degree of trauma (21) Burns are easily classified with regard to the degree of injury, and extensive burns are, according to Moore (21), the severest form of trauma experienced by man It follows that burned patients are particularly suitable for the study of metabolic changes produced by severe trauma This study showed that the changes in lipid metabolism were also well correlated to the degree of trauma The more extensive the burn, the higher and more sustained was the initial increase in FFA Similarly the decrease of cholesterol and phospholipids was greater and persisted longer in the more severe burns Since the changes in the different plasma lipids characteristically occurred at different phases of the burn, they will be discussed separately below

Plasma FFA

It appears likely that the increased concentration of FFA in plasma after trauma is due to an increased rate of mobilization of FFA from adipose tissue First, increased levels of FFA in different conditions are generally due to a stimulation of the mobilization into plasma and seldom due to a reduced fractional turnover rate in plasma Secondly, Moore has shown that adipose tissue — the major precursor of plasma FFA — decreases very significantly after burns (21) It has been pointed out that oxidation of endogenous fat replaces oxidation of ingested carbohydrate as a source of

bodily energy and that this change represents one of the basic metabolic consequences of trauma (21) In this series the highest value of FFA in all three groups was found during the first three days after burn, that is during the shock and acute fluid imbalance period During this period all the patients had satisfactory circulatory and kidney function

The two major factors which may be responsible for an enhanced mobilization of FFA from adipose tissue in trauma are the deficiency of calories and the increased activity of the sympathetic nervous system During the first week the supply of calories was slightly higher in group 3 than in group 2 As the fasting FFA level was higher in group 3 than in group 2 this suggests that factors other than the caloric supply also were of importance for the regulation of the FFA level It is of interest in this connection that Wadstrom (25) showed that the increase in plasma FFA on the day after a cholecystectomy exceeded that attributable to the low caloric supply

It was previously found (1 2) that the urinary excretion of catecholamines was elevated during the first two weeks in patients with medium sized and extensive burns In small burns the excretion of catecholamines was elevated only during the first three days The more extensive the burn the higher and more sustained was the rise in the excretion of catecholamines The same tendency was found for the FFA level in the different groups in this study This relationship suggests that the activity of the sympathetic nervous system after trauma is important in stimulating mobilization of FFA and thereby elevating their plasma level In support of this, we demonstrated earlier that treatment of dogs with guanethidine, a peripherally acting sympathetic inhibitor, inhibited the post traumatic rise in plasma FFA and the simultaneous occurring increase of liver triglycerides (9)

Increased mobilization of FFA after burns may be of value in supplying lipids for oxidation on various cells of the body, as the caloric supply is low in this situation However, if the mobilization of FFA exceeds the oxidative needs of the body this may induce pathological changes in the body, e g lipid deposition in various organs and increase in the basal metabolic rate (11)

Excessive mobilization of FFA during infusion of noradrenaline in the dog is followed by increased lipid deposition in various organs and raised body temperature (11, 12, 16) Gillman and Gillman (17) described fatty degeneration of the liver cells in burned patients who died during the first two weeks after the accident Two of our cases who died on day 8 and 10 had also, according to the pathologists fatty livers Increased metabolic rate has been observed several weeks after severe burns (2 13) Most of the burn patients have a slowly rising temperature in the early days without signs of infection This rise can at least in part be due to increased mobilization and oxidation of FFA.

Plasma cholesterol and phospholipids

These lipid classes followed each other closely which indicates that the major effect of the trauma was on the intire lipoprotein molecules (low density and

high density) which carry these lipids in plasma. The analysis of the lipoproteins, however, revealed that changes occurred also in the composition of the lipoproteins, as will be discussed below.

Dodds and Mills (14) show that during myocardial infarction the cholesterol level in plasma decreases during the initial phase with a lowest level occurring after about one week, similarly to the results of this study. Landon et al (20) has demonstrated that the cholesterol level decreases in patients with inflammatory polyarthritis, and the level was found to be related to the severity of the disease. Low serum-cholesterol values were also found in inflammatory pulmonary disease. Interestingly, Guravich and Venegas in 1962 (18) reported the fortuitous observation that a case of essential hypercholesterolemia had a decrease in plasma cholesterol to 312 mg per 100 ml followed by a rebound to 556 after a burn accident.

The decrease of cholesterol we observed was most pronounced at the time when signs of liver damage were present. Reichard et al (22) show that burned patients had increased levels of enzymes (GOT, GPT, OCT) in plasma, indicative of liver injury. This increase was most marked 9 days after the injury. The enzyme levels returned to normal 2—3 weeks later, which indicated that the liver injury was reversible. These patients received the same treatment as the patients of this study.

The plasma lipoproteins, the carriers of cholesterol and phospholipids in plasma, are known to be synthesized in the liver. A decreased hepatic synthesis of the plasma lipoproteins could well be the explanation of the low levels of cholesterol and phospholipids. Increased rates of elimination of the lipoproteins from the plasma compartment by leakage and exudation of the lipoproteins through the burned area might also be involved in causing the decreased concentration. This latter possibility cannot, of course, hold for other stress conditions such as myocardial infarction or inflammatory diseases. The correlation between cholesterol and albumin in the plasma changes during the first 6 weeks after the trauma suggests that the same mechanism may be responsible for the changes in these plasma constituents. The low albumin levels in inflammatory diseases are possibly caused by a decreased synthesis of albumin (19). In severe burns there is an increased catabolism of albumin for several weeks after the accident (3). The synthesis of albumin is not capable of compensating for the increased catabolism during this period. It is possible that, in this situation, the flow of amino acids for plasma protein synthesis is directed into albumin synthesis and diverted from other plasma proteins such as the lipoproteins. This could explain the correlation between the concentrations of albumin and cholesterol in plasma.

It is noteworthy that the concentrations of cholesterol and phospholipids on the day of admission were the same as when the burn was healed after 6 to 12 months. At that time the patients were in a good nutritional state and had resumed their ordinary diets. It has been debated whether the blood lipid values of patients with myocardial infarction on the day of the infarction are representa-

tive or not for these patients' pre infarc tion level This question has, however, been difficult to assess since these patients may for several reasons change their diets The present study trongly suggests that the values for at least cholesterol and phospholipids on the day of the trauma are representative of the pre traumatic level in the patients

Plasma lipoproteins

The data show that the plasma concen trations of the high and low density lipoproteins were similarly influenced by the trauma during the first 7 to 10 days Consideration of the great differences between these lipoprotein species with regard to lipid composition and molecu lar size suggests that the effect was not mediated via an effect on the lipid metabolism or on the removal of these lipoproteins from plasma One possible common cause may be through an effect on the synthesis of the protein part of lipoproteins in the liver The more rapid return to normal of the concentration of the high density than of the low density lipoproteins indicates different mecha nism of control for the metabolism of these two major plasma lipoprotein frac tions

Summary

The concentrations of free fatty acids (FFA) cholesterol phospholipids and triglycerides in plasma were followed in 38 burned patients from the beginning of the trauma up to 6 to 12 months afterwards

The concentration of FFA was elevated initially The more pronounced the trauma the greater and more prolonged was this elevation The FFA level could not be directly correlated to the caloric intake The role of increased activity of the sympathetic nervous system in this elevation was discussed

The cholesterol and phospholipid levels decreased during the first 7—10 days after the trauma, then increased slowly and were not restored to the initial value until after 6 to 12 months The greater the trauma, the greater was the effect on the concentration of cho lesterol and phospholipids

The concentration of triglycerides showed no statistically significant changes during the course of burn

There was a strong correlation between the changes in concentrations of cholesterol and albumin during the first 6 weeks after the trauma The role of the liver in these changes was discussed

The lipid composition of the plasma lipoproteins, separated in the preparative ultracentrifuge was followed in 6 patients with major burns During the first 7—10 days there was a similar fall in the concentration of cholesterol and phospholipids in the high and low density lipoproteins After about 4 weeks the lipid content of the high density lipoproteins had been restored However, at that time the low density lipoprotein class contained only about 55 per cent of its initial content of cholesterol and phospholipids The qualitative composition of the high and low density lipoproteins was also changed significantly after the trauma Thus the cholesterol/phospholipids ratio had after one week decreased from 0 45 and 1 36 to 0 33 and 1 00 for the high and low density lipoproteins, respectively

Acknowledgement

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Glucose Metabolism in Thyroid Disease

By

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There is a vast body of information about glucose metabolism in experimental and clinical hyper and hypo-thyroidism but the results reported are in many respects conflicting This applies especially to studies made on man In animal experiments it was long ago shown that an excess of thyroid hormones induces an increase in the tissue utilization of glucose and increased disappearance of glucose from the blood with depletion of the glycogen stores in the liver In man such studies have been mainly confined to the analysis of the blood glucose after oral or intravenous glucose administration with very divergent results (cf 16) The present study was carried out in the expectation that the combined use of three tests — intravenous glucose tolerance, glucagon and tolbutamide — might provide some data of interest concerning glucose metabolism in clinical hyper and hypo-thyroidism

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Material and methods

The series of patients studied comprised 23 hyperthyroid (cases 1—23) 5 hypothyroid (cases 24—28) and 28 euthyroid subjects In addition 11 hyperthyroid cases were studied with special reference to glucosuria occurring during the intravenous glucose tolerance test. Of the hyperthyroid cases about half had Graves disease superimposed on endemic nodular goitre as judged from the thyro-hypophysial eye signs of true exophthalmos (3) and the other half had nodular toxic goitre without eye signs The *euthyroid subjects were mainly young or* middle aged people suffering from congenital heart disease without congestive heart failure and hospitalized for heart catheterization A few patients were moderately obese without any evidence of endocrine disorders The mean age in the hyperthyroid group was 39 4 years and in the control group 29 7 years

I v glucagon test 1 mg of glucagon (Lilly) diluted in 150 ml of physiological saline was *infused intravenously* during 60 min Capillary blood was drawn before and 15 30 45 60 75 90 120 and 180 min after starting the infusion

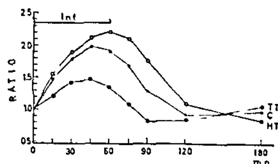


Fig 1 The changes in blood glucose concentration during and after infusion (Inf) of 1 0 g of glucagon in hyperthyroid (TT) hypothyroid (HT) and control (C) subjects The values from 15 to 90 min in the hyperthyroid group are statistically significantly different from the corresponding values in the control group

I v tolbutamide test 1 0 g of sodium tolbutamide in 20 ml of sterile water was injected intravenously within a few minutes Capillary blood was drawn before and 15, 30, 45, 60 and 120 min after injection

I v glucose tolerance test 25 0 g of glucose diluted in 100 ml of sterile water was injected intravenously within 3—4 min Capillary blood was drawn before and 10, 20 30, 40 50 and 60 min after the commencement of the injection

The glucose concentration in the capillary blood was determined enzymatically

In the glucagon and tolbutamide tests the glucose concentration at different times was expressed as a ratio of the fasting level

The glucose disappearance rate constant k was calculated from the slope of the disappearance curve for each subject separately by the method of least squares (7 10, 27, 33) All values from 10 min to 60 min were used except when the fasting level was reached before 60 min had elapsed This happened in a few hyperthyroid subjects From the curve so obtained the zero intercept was extrapolated The *apparent distribution volume of glucose* was calculated in the following manner the fasting concentration (C_f) and the concentration at the zero intercept (C_i) were corrected for blood water by dividing them by 0 83 (38) and the difference between the two values ($C_{i\,corr}—C_{f\,corr}$) calculated The distribution volume in litres was obtained by dividing the amount of glucose administered (25,000 mg) by $C_{i\,corr}—C_{f\,corr}$, expressed in mg/l The *absolute glucose utilization* was obtained by multiplying the dose administered (25,000 mg) by the rate constant k, the result being expressed in mg/min This value was further related to the body surface area as determined from the height and weight of the patient (44), the final value so obtained expressed the glucose utilization in mg min^{-1} m^{2} 1

None of the subjects studied had spontaneous glucosuria The loss of glucose in the urine during the i v glucose-tolerance test is negligible (38) During the actual experiments glucosuria was not determined Hence 11 additional cases with hyperthyroidism were studied in this respect In 10 of them the glucosuria varied from 0 to 404 mg during 60 min, with a mean of 98 mg In the eleventh case the glucosuria was 2080 mg but when an oral glucose tolerance test was performed a few days later no glucosuria appeared and the blood glucose curve was not of the diabetic shape At all events, the loss via the urine would not profoundly influence the glucose disappearance rate in the blood

Results

I v glucagon test The results are collated in table I and fig 1 In the control subjects the peak of the glucose curve appeared about 45 min after commencement of the infusion and the maximal concentration was about twice the fasting level In the hyperthyroid subjects the maximal rise appeared at about the same time but was only 50 per cent of the fasting concentration All the values between 15 and 90 min after the start of the infusion were significantly below the corresponding values in the control group ($p < 0\ 001$) The initial

TABLE I Changes in the blood glucose level during and after intravenous infusion (60 min) of 1 mg glucagon

Case No	C_f[1]	Minutes after commencement of infusion[2] 15	30	45	60	75	90	120	180
Hyperthyroidism									
1	84	1 357	1 548	1 702	1 429	0 905	0 548	0 833	–
2	85	1 060	1 329	1 518	1 612	1 494	1 259	0 776	1 060
3	77	1 221	1 532	1 494	1 429	0 974	0 662	0 870	1 039
4	79	1 316	1 519	1 405	1 101	0 810	0 558	0 658	1 000
5	87	1 310	1 632	1 782	1 609	1 103	0 782	1 000	1 126
6	85	1 118	1 306	1 329	1 141	0 882	0 471	0 706	0 882
7	76	1 250	1 300	1 632	1 684	1 553	1 342	0 961	1 184
8	75	1 373	1 573	1 747	1 613	1 427	0 920	0 733	1 000
9	97	1 155	1 268	1 000	0 825	0 691	0 629	0 866	0 969
10	77	1 260	1 532	1 416	1 416	1 130	0 727	0 636	1 065
11	87	1 069	1 184	1 437	1 368	1 103	0 805	0 839	1 115
12	85	1 247	1 329	1 330	1 247	0 965	0 765	0 718	1 000
13	82		1 329		1 268		0 902	0 927	1 000
14	90		1 422		1 356		1 267	1 010	1 278
15	73		1 713		1 438		0 836	0 918	1 000
16	75	1 200	1 347	1 520	1 360	1 200	0 747	0 990	1 147
17	74	1 189	1 351	1 378	1 149	0 905	0 689	0 703	–
Mean		1 223	1 436	1 478	1 356	1 082	0 818	0 832	1 058
P		<0 001	<0 001	<0 001	<0 001	<0 001	<0 001	<0 001	<0 05
±S D		0 0026	0 0351	0 0543	0 0536	0 0696	0 0619	0 0302	0 0255
Hypothyroidism									
24	89	1 213	1 393	1 618	1 640	1 596	1 371	0 742	0 663
25	72	1 514	2 014	2 125	2 361	2 319	1 972	1 417	1 000
26	59	1 695	1 847	1 812	1 812	1 627	1 237	0 525	–
27	60	1 817	2 367	2 950	3 083	2 917	2 500	1 700	0 817
Mean		1 560	1 905	2 126	2 224	2 115	1 770	1 036	0 827
±S D		0 1313	0 2023	0 2938	0 3250	0 3151	0 2912	0 2768	0 0974
Control subjects									
29	76	1 092	1 487	1 868	2 092	1 961	1 447	0 961	0 855
30	83	1 651	1 892	2 096	2 361	2 229	1 880	1 410	1 010
31	69	1 696	2 090	2 043	1 971	1 319	0 942	0 768	1 058
32	74	1 622	2 000	2 149	1 838	1 243	1 135	0 865	0 932
33	61		2 000		1 656		1 246	0 770	0 934
34	80	1 338	1 550	1 575	1 613	1 288	1 000	0 713	1 000
35	67	1 462	1 750	1 960	1 910	1 790	1 420	0 778	1 090
36	80		1 675		1 625		0 825	0 864	1 010
37	70	1 43	1 81	2 130	2 060	1 850	1 430	0 858	0 958
38	78	1 580	1 65	2 06	2 110	1 910	1 650	1 150	0 855
Mean		1 484	1 790	1 935	1 924	1 699	1 298	0 914	0 970
±S D		0 0 06	0 0644	0 0670	0 0775	0 1300	0 1046	0 0677	0 0249

[1] fasting glucose level mg/100 ml [2] – ratio to C_f

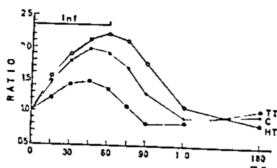


Fig. 1. The changes in blood glucose concentration during and after infusion (Inf.) of 1.0 g of glucagon in hyperthyroid (TT), hypothyroid (HT) and control (C) subjects. The values from 15 to 90 min. in the hyperthyroid group are statistically significantly different from the corresponding values in the control group.

I. v. tolbutamide test. 1.0 g of sodium tolbutamide in 20 ml of sterile water was injected intravenously within a few minutes. Capillary blood was drawn before and 15, 30, 45, 60 and 120 min. after injection.

I. v. glucose-tolerance test. 25.0 g of glucose diluted in 100 ml of sterile water was injected intravenously within 3—4 min. Capillary blood was drawn before and 10, 20, 30, 40, 50 and 60 min. after the commencement of the injection.

The glucose concentration in the capillary blood was determined enzymatically.

In the glucagon and tolbutamide tests the glucose concentration at different times was expressed as a ratio of the fasting level.

The glucose disappearance rate constant k was calculated from the slope of the disappearance curve for each subject separately by the method of least squares (7, 10, 27, 33). All values from 10 min. to 60 min. were used except when the fasting level was reached before 60 min. had elapsed. This happened in a few hyperthyroid subjects. From the curve so obtained the zero intercept was extrapolated. The *apparent distribution volume of glucose* was calculated in the following manner: the fasting concentration (C_f) and the concentration at the zero intercept (C_i) were corrected for blood water by dividing them by 0.83 (38) and the difference between the two values ($C_{i\,corr}—C_{f\,corr}$) calculated. The distribution volume in litres was obtained by dividing the amount of glucose administered (25,000 mg) by $C_{i\,corr}—C_{f\,corr}$ expressed in mg/l. The *absolute glucose utilization* was obtained by multiplying the dose administered (25,000 mg) by the rate constant k, the result being expressed in mg/min. This value was further related to the body surface area as determined from the height and weight of the patient (44), the final value so obtained expressed the glucose utilization in mg min^{-1} m^{2} ¹.

None of the subjects studied had spontaneous glucosuria. The loss of glucose in the urine during the i. v. glucose-tolerance test is negligible (38). During the actual experiments glucosuria was not determined. Hence 11 additional cases with hyperthyroidism were studied in this respect. In 10 of them the glucosuria varied from 0 to 404 mg during 60 min., with a mean of 98 mg. In the eleventh case the glucosuria was 2080 mg, but when an oral glucose-tolerance test was performed a few days later no glucosuria appeared and the blood glucose curve was not of the diabetic shape. At all events, the loss via the urine would not profoundly influence the glucose disappearance rate in the blood.

Results

I. v. glucagon test. The results are collated in table I and fig. 1. In the control subjects the peak of the glucose curve appeared about 45 min. after commencement of the infusion and the maximal concentration was about twice the fasting level. In the hyperthyroid subjects the maximal rise appeared at about the same time but was only 50 per cent of the fasting concentration. All the values between 15 and 90 min. after the start of the infusion were significantly below the corresponding values in the control group ($p < 0.001$). The initial

TABLE I Changes in the blood glucose level during and after intravenous infusion (60 min) of 1 mg glucagon

Case		Minutes after commencement of infusion*							
No	C_f[1]	15	30	45	60	75	90	120	180
Hyperthyroidism									
1	84	1 357	1 548	1 702	1 429	0 905	0 548	0 833	~
2	85	1 060	1 329	1 518	1 612	1 494	1 259	0 7 6	1 060
3	77	1.221	1 532	1 494	1 429	0 974	0 662	0 8,0	1 039
4	79	1 316	1 519	1 405	1 101	0 810	0 558	0 658	1 000
5	87	1 310	1 632	1 ,82	1 609	1 103	0 782	1 000	1 126
6	85	1 118	1 306	1 329	1 141	0 882	0 471	0 706	0 882
7	76	1 250	1 500	1 632	1 684	1 553	1 342	0 961	1 184
8	75	1 373	1 573	1 747	1 613	1 427	0 920	0 733	1 000
9	97	1 155	1 268	1 000	0 825	0 691	0 629	0 866	0 969
10	77	1.260	1 532	1 416	1 416	1 130	0 727	0 636	1 065
11	87	1 069	1 184	1 437	1 368	1 103	0 805	0 839	1 115
12	85	1 247	1 329	1 330	1 247	0 965	0 ~65	0 718	1 000
13	82		1 329		1 268		0 902	0 927	1 000
14	90		1 422		1 356		1 267	1 010	1 278
15	73		1 713		1 438		0.836	0 918	1 000
16	75	1 200	1 347	1 520	1 360	1 200	0 747	0 990	1 147
17	74	1 189	1 351	1 378	1 149	0 905	0 689	0 703	~
Mean		1 223	1 436	1 478	1 356	1 082	0 818	0 832	1 058
p		<0 001	<0 001	0 001	<0 001	<0 001	0 001	<0 001	<0 05
±S D		0 0026	0 0351	0 0543	0 0536	0 0696	0 0619	0 0302	0 0255
Hypothyroidism									
24	89	1 213	1 393	1 618	1 640	1 596	1 371	0 742	0 663
25	72	1 514	2 014	2 125	2 361	2 319	1 972	1 417	1 000
26	59	1 695	1 847	1 812	1 812	1 62	1 237	0 525	
27	60	1 817	2 367	2 950	3 083	2 917	2 500	1 00	0 817
Mean		1 560	1 905	2 126	2 224	? 115	1 770	1 096	0 827
S D		0 1313	0 2023	0 2938	0 3250	0 3151	0 2912	0 2 F8	0 0974
Control subjects									
29	6	1 092	1 487	1 868	2 092	1 961	1 447	0 961	0 855
0	83	1 651	1 892	2 096	2 361	2 229	1 880	1 410	1 010
31	69	1 696	2 030	2 043	1 371	1 319	0 942	0 68	1 058
32	74	1 622	2 000	2 149	1 838	1 243	1 135	0 865	0 932
33	61		2 000		1 656		1 216	0 770	0 934
34	80	1 338	1 550	1 575	1 613	1 288	1 000	0 713	1 000
35	67	1 462	1 750	1 960	1 910	1 790	1 420	0 778	1 090
36	80		1 675		1 625		0 825	0 864	1 010
3	0	1 43	1.81	2 130	2 0F0	1 850	1 430	0 8.8	0 958
38	78	1 580	1 65	2 06	2 110	1 910	1 650	1 150	0 855
Mean		1 484	1 790	1 985	1 924	1 699	1 298	0 914	0 970
±S D		0 0706	0 0644	0 06 0	0 0775	0 1300	0 1046	0 0677	0 0249

[1] fasting glucose level mg/100 ml * ratio to C_f

TABLE II Changes in blood glucose level after intravenous injection of 1 mg tolbutamide

Case No	C_f[1]	Minutes after injection[2] 15	30	60	120
Hyperthyroidism					
1	87	0 721	0 540	0 666	0 795
2	97	0 865	0 620	0 826	0 743
3	85	0 517	0 611	0 765	0 965
4	69	0 508	0 406	0 782	0 914
5	81	0 765	0 703	0 840	1 070
6	79	0 735	0 430	0 761	0 988
7	69	0 770	0 666	0 900	0 941
8	84	0 725	0 682	0 740	0 952
9	96	0 550	0 605	0 824	1 050
10	92	0 816	0 652	0 630	0 936
11	90	0 634	0 512	0 734	0 920
12	93	0 754	0 601	0 570	0 880
13	88		0 522	0 715	0 828
14	92		0 782	0 685	0 904
15	78		0 744	0 833	1 010
16	82	0 610	0 512	0 744	0 842
17	77	0 662	0 546	0 700	1 000
Mean		0 687	0 596	0 748	0 926
p		<0 001	<0 05		
±S D		0 0296	0 0252	0 0203	0 0214
Hypothyroidism					
24	77	0 961	0 780	0 780	0 961
25	61	0 935	0 868	0 762	0 802
27	61	0 836	0 736	0 690	0 916
Mean		0 911	0 795	0 744	0 893
p			0 02		
±S D		0 0381	0 0388	0 0275	0 0473
Control subjects					
29	66	0 740	0 560	0 638	0 850
30	84	0 952	0 680	0 702	0 725
32	78	0 820	0 680	0 602	0 745
33	66	—	0 652	0 880	1 000
34	72	0 890	0 710	0 654	0 848
35	75	0 850	0 710	0 650	0 840
37	80	0 77[illegible]	0 675	0 600	0 775
38	84	0 930	0 665	0 630	0 870
39	64	1 010	0 810	0 9[illegible]0	1 030
40	60	0 820	0 750	0 820	1 050
41	100	0 820	0 560	0 760	0 840
42	60	0 783	0 610	0 800	1 020
Mean		0 854	0 672	0 726	0 883
±S D		0 0250	0 0209	0 0346	0 0330

[1] C_f = fasting glucose level, mg/100 ml [2] = ratio to C_f

level was again reached at 90 min, whereas this happened in the control group only at 120 min In the hypothyroid group the increase was greater than in the control group and the maximal rise seemed to occur somewhat later, but owing to the small number of cases studied the differences were not statistically significant No major distress was experienced in general by the patients, except for slight hypoglycaemia symptoms 1—2 hrs from the start of the infusion In one hypothyroid patient, however, a prolonged hypoglycaemia developed after 3 hrs that lasted for about 2 days (case 26)

I v tolbutamide test The results are shown in table II and fig 2 In the control group there was a linear drop in the glucose concentration with a minimal value 30 min after the injection In the hyperthyroid group the decrease was significantly faster and the minimal value reached at 30 min was lower than in the control group After 15 and 30 min the values obtained in the hyperthyroid group were statistically significantly lower than in the control group ($p < 0\ 001$ and $< 0\ 05$ resp) In the hypothyroid subjects the decrease was more sluggish and the maximal decrease only occurred after 60 min The 30 min value was significantly higher than the corresponding value in the control group ($p < 0\ 02$) After 60 min a gradual parallel increase occurred in all groups of patients

The *fasting glucose level* (table IV) was 76 3 mg/100 ml in the control group 83 0 mg/100 ml in the hyperthyroid and 64 6 mg/100 ml in the hypothyroid group The differences between the

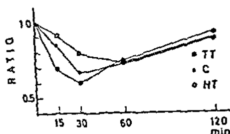


Fig 2 The changes in blood glucose concentration after intravenous injection of 1 0 g of tolbutamide in hyperthyroid (TT) hypothyroid (HT) and control (C) subjects. The values at 15 and 30 min. in the hyperthyroid and that at 30 min in the hypothyroid group are statistically significantly different from the corresponding values in the control group

groups were statistically significant ($p < 0\ 001$)

I v glucose tolerance test The results are shown in tables III and IV The *glucose disappearance rate constant k*, expressed as per cent/min (k) was 1 63 in the hyperthyroid 1 43 in the control and 1 14 in the hypothyroid group and accordingly the half time was 42 9, 48 3 and 61 1 min, respectively Only the difference between the hyperthyroid and the hypothyroid group was statistically significant ($p < 0\ 01$) The calculated *absolute glucose utilization* was 300 mg per min per square metre in the hyperthyroid 225 mg in the control and 180 mg in the hypothyroid group Only the difference between the hyperthyroids and the controls was statistically significant ($p < 0\ 05$) The *zero intercept* extrapolated from the glucose disappearance curve was 241 2 mg/100 ml for the hyperthyroid 229 5 mg/100 ml for the control and 208 0 mg/100 ml for the hypothyroid group The differences between the groups were statistically significant The differences were apparently

TABLE III Results of the intravenous glucose-tolerance test in hyperthyroidism and hypothyroidism

Case No	Age sex	PBI	C_f	C_i	$T^{1/2}$ (min)	k 10³ (%/min)	Gu	BSA
Hyperthyroidism								
1	61♀	9 0	105	322	52 1	1 33	227 7	1 46
2	58♀	18 4	95	234	50 3	1 37	231 4	1 48
3	42♀	9 0	83	247	36 0	1 93	294 2	1 64
4	60♀	8 3	71	255	21 1	3 28	494 0	1 66
5	18♂	13 4	86	189	72 9	0 95	127 0	1 87
6	32♀	13 9	83	259	26 4	2 63	416 1	1 58
7	33♀	13 4	90	243	55 7	1 25	183 8	1 70
8	33♀	12 5	74	241	48 3	1 43	193 2	1 85
9	35♀	13 8	89	252	47 9	1 45	241 7	1 50
10	60♀	12 0	61	230	65 8	1 05	165 1	1 59
11	38♀	10 7	86	240	31 2	2 22	338 4	1 64
12	34♂	13 1	93	202	33 6	2 06	264 1	1 95
13	54♀	14 1	83	259	41 2	1 68	264 2	1 59
14	57♀	12 8	93	216	47 0	1 48	206 7	1 79
15	50♀	11 1	75	269	27 6	2 51	354 5	1 77
16	24♀	11 6	80	303	14 4	4 81	840 9	1 43
17	24♂	11 0	79	230	41 1	1 69	232 1	1 82
18	26♀	10 2	71	249	35 8	1 93	294 2	1 64
19	42♀	10 0	82	192	47 1	1 47	213 7	1 72
20	18♀	18 0	73	192	54 0	1 28	228 6	1 40
21	16♀	18 2	78	227	27 3	2 54	378 0	1 68
22	45♀	12 5	94	238	41 8	1 66	244 1	1 70
23	47♀	11 2	77	316	22 3	3 10	458 6	1 69
Hypothyroidism								
24	57♂	1 9	71	238	49 4	1 40	221 5	1 58
25	50♀	1 3	64	234	73 9	0 94	149 7	1 58
27	25♀	1 2	61	202	51 6	1 34	214 7	1 56
28	17♂	0 8	35	166	80 9	0 86	135 2	1 59

PBI μg/100 ml
C_f = fasting glucose level mg/100 ml
C_i = zero intercept mg/100 ml
Gu = glucose utilization mg/min/m²
BSA = body surface area m²

not due to variations in the *distribution volume* (13 5 litres in the hyperthyroid, 14 2 in the control and 13 8 in the hypothyroid subjects, the differences being without significance), but evidently reflected only the differences in the fasting glucose level The distribution volume was also correlated with the body surface area but no significant difference could be detected between the groups

Discussion

It has been shown in animal experiments that the glycogen stores of the liver are depleted during treatment with thyroid hormones (11, 14, 17, 30, 43), and this has also been assumed to be the case for clinical hyperthyroidism in man The hyperglycaemic response to i v glucagon in the present study was significantly less in the hyperthyroid than in the control subjects Possible explanations of this finding are a) that the enzymic apparatus responsible for liberation of glucose from glycogen does not respond in a normal way b) that the glucagon infused is degraded much faster in hyperthyroidism than in normal people, c) that the glucose liberated is removed much faster in hyperthyroidism or, d) that the glycogen stores are depleted Most of the evidence points in favour of the last mentioned view Hyperthyroid patients have a tendency to ketoacidosis on fasting (28) and there is a significant elevation of the fasting blood glucose level (6 16 41), also confirmed here An increase of the glucose 6 phosphate dehydrogenase activity in the liver has been observed in experimental hyperthyroidism (20) and in clinical thyrotoxicosis a similar, although not significant trend has been observed (39) All these facts taken together are compatible with the idea that there is no defect in the enzymes but that the glycogen stores are diminished The removal of glucose from the blood is not accelerated to such an extent that this could explain the low response to glucagon and it is hardly credible that the degradation of glucagon could be accelerated to such an extent Ungar et al (45) recently suggested that glucagon is a hormone that responds to the glucose need in the peripheral tissues, and the need may indeed by increased in hyperthyroidism The reverse would seem logically be likely in hypothyroidism there was an opposite trend in the hypothyroid group although the change was not statistically significant, probably because the number of cases studied was too small

Glucose utilization has been shown to be increased in experimental hyperthyroidism, decreased in hypothyroidism and restored to normal by treatment with thyroid hormones (22, 37, 42) Similar findings have also been made in tissues isolated from animals pretreated with thyroid hormones (5) The situation in man is still under debate Studies with intravenous and oral glucose tolerance tests have yielded rather conflicting results, which has been thought to be partly due to the methods by which the data have been treated The subject has been thoroughly reviewed by Elrick et al (16) However Sanger & Hulin (41) found that in clinical hyperthyroidism glucose utilization as calculated from the respiratory quotient was significantly elevated The arteriovenous difference is high in contrast to diabetes (28) Oral glucose tolerance tests have however given results that have sometimes been interpreted as being of diabetic type but it is also well known that there is a considerable variation (4 6 12 19, 21, 23, 29 40) It has been claimed that in hyperthyroidism there is an increased absorption of glucose from the intestine (3) which is probably the reason for the

TABLE IV. Mean values and statistical evaluation of the i.v. glucose tests

Group	No.	C_f	C_i	C_i-C_f	C_i-C_f corr.
Hyperthyroidism	23	82.95 ±1.132	241.2 ±2.0	158.5	191.0
Hypothyroidism	4	64.55 ±4.059	203.0 ±3.6	150.2	181.0
Controls	28	76.28 ±1.293	229.5 ±1.5	151.4	182.4
P Hyperthyroidism vs. controls		<0.001	<0.001		
P Hypothyroidism vs. controls		<0.001	<0.001		
P Hyperthyroidism vs. hypothyroidism		<0.001	<0.001		

C_f = fasting glucose level, mg/100 ml.
C_i = zero intercept, mg/100 ml.
C_i-C_f corr. = corrected for blood water content.

type of curve observed in hyperthyroidism, usually showing a high peak about 1 hour after ingestion of the glucose. The removal of glucose from the blood after intravenous administration also shows a great variation in clinical hyperthyroidism, being usually normal (4, 16, 32, 34) but sometimes accelerated (4). The manner in which i.v. glucose data should be analysed has also been a matter of debate (4, 16). Elrick et al. (16) used a constant i.v. infusion of glucose and found no difference between hyperthyroid and control subjects but a significant decrease in glucose disappearance in hypothyroidism. In many cases a reduction in the rate of fall has been observed when hyperthyroid patients have been brought to a euthyroid level (4). A decreased utilization rate was also observed in hypothyroidism (16, 34).

In the present study there was no significant difference between groups as far as the *glucose disappearance rate constant*, *k*, was concerned, although the means differed to some extent except when the hyperthyroid group was compared with the hypothyroid one. This would in any case indicate that a difference exists when the change in thyroid hormone level is sufficiently large, and only then becomes measurable by this method. It is noteworthy that Christophe et al. (8) observed a significant acceleration of the disappearance rate in animals after a single injection of thyroxine, but that when the animals were treated for one week the disappearance rate was normalized, indicating the activation of adjusting mechanisms during prolonged treatment. The same is probably the case in clinical hyperthyroidism. When the *absolute glucose utilization* per minute per square metre of body sufrace was calculated, however, there was a significant elevation in the hyperthyroid group for

Vol (l)	$T^1/_2$ (min)	k 10^2 (%/min)	Gu	BSA	V/BSA
13.5	42.96	1.613 ±0.114	299.7 ±31.4	1.659	8.15
13.8	61.06	1.135 ±0.139	180.0 ±22.2	1.575	8.78
14.2	48.25	1.437 ±0.077	224.9 ±15.5	1.738	8.19
n.s.		>0.05	<0.05		n.s.
n.s.		>0.05	>0.05		n.s.
n.s.		<0.001	>0.05		n.s.

Gu = glucose utilization mg/min/m^2
BSA = body surface area m^2
n.s. = not significant.

which the value was 300 mg against 225 mg in the controls. Again the hypothyroid group was rather small but Macho (34) referring results to body weight, observed a significant decrease in hypothyroidism but no difference in hyperthyroidism.

More than a half century ago alimentary glucosuria was reported to be frequent in hyperthyroidism. This is believed to correspond to the high peaks after ingestion of carbohydrates. None of the patients studied had spontaneous glucosuria. In 10 cases the glucosuria during the i.v. glucose tolerance test varied from 0 to 404 mg in one hour with a mean of 98 mg; in the eleventh case it was about 2 000 mg but it may be of significance as regards the glucosuria in hyperthyroidism that when an oral glucose tolerance test was performed in this case a few days later no glucosuria appeared although the blood curve showed a typical high peak. The range of glucosuria during the test corresponds well to the values observed by Macho and fall well within the normal range (34). The loss of glucose in the urine during the tests would not significantly influence the glucose disappearance curve for the blood.

The responsiveness to insulin in thyroid disorders has been found to be rather variable (1, 29, 35, 36). Recently however, Danowski et al. (13) reported on an increased responsiveness to tolbutamide in normal subjects treated with an excess of thyroid hormones. In the present study the response to *i.v. tolbutamide* was significantly faster and more marked in hyperthyroid subjects than in euthyroid controls and *significantly less* in hypothyroid patients. The reason for this may be complex, as pointed out by Danowski (12), but the possibility of hyperinsulinization is of great in

terest in appraisal of the relations between hyperthyroidism and diabetes mellitus The incidence of diabetes mellitus is higher in hyperthyroidism than in the average population (28, 29) The literature on this subject has been recently thoroughly reviewed by Abt (2) There is some evidence of increased degradation of insulin in hyperthyroidism (9, 15, 16) Although these observations require confirmation, the fact in itself seems not very surprising, since the increase in general catabolism in hyperthyroidism is well known Very few studies on the plasma level of insulin have been made so far and the results are too scanty to be adequately evaluated (21, 46) The fact that thyroid and metathyroid diabetes can be induced in animals after partial pancreatectomy (24, 25) suggests that an excess of thyroid hormones, indeed, places some strain on carbohydrate metabolism and on the mechanism by which this is controlled Hence it would seem conceivable that clinical hyperthyroidism results in an increase in glucose utilization which in turn induces depletion of the glycogen stores and possibly hyperinsulinization' through a constant elevation of the fasting glucose level in the blood The alimentary carbohydrate peaks and an increased degradation of insulin might be additional influences all these factors together having in the long run a destructive effect on the insulin-producing mechanism which in animal experiments shows signs of degeneration (25) or hyperplasia (17) One point which seems to be of considerable interest but requires confirmation is the observation of Gedda (18) that TSH may increase the blood glucose level What bearing this finding may have on the present problems is still difficult to visualize

As to the diagnosis of diabetes in hyperthyroidism, long ago it was pointed out (23) that the usual criteria are not valid It would seem, in the light of the present observations, that the i v glucose tolerance test and the i v tolbutamide test will prove excellent tools for this differentiation

Summary

Some aspects of glucose metabolism were studied in 23 hyperthyroid, 6 hypothyroid and 28 control subjects by means of i v glucagon infusion, i v tolbutamide injection and i v glucose tolerance tests

I v infusion of 1 mg of *glucagon* induced a significantly lower response in the hyperthyroid than in the control subjects, the maximal increases in blood glucose being 18 and 99 per cent of the fasting level, respectively In hypothyroidism the opposite trend was observable although the difference was not significant I v injection of 1 g of *tolbutamide* brought about a significantly faster and enhanced decrease in the blood glucose level in the hyperthyroid and a significantly more sluggish effect in the hypothyroid subjects than in the control cases The *glucose disappearance rate constant* was 1 61 in the hyperthyroid 1 14 in the control and 1 14 in the hypothyroid subjects only the differences between the hyperthyroid and hypothyroid groups being significant The fasting glucose level was significantly higher in

the hyperthyroid and significantly lower in the hypothyroid groups than in the controls The *absolute glucose utilization* was 300 mg/min/m² of the body surface area in the hyperthyroid, 225 mg in the controls and 180 mg in the hypothyroid cases The difference between hyperthyroid and control subjects was significant

Spontaneous glucosuria was not seen in the 23 and in 11 additional hyperthyroid cases Glucosuria appeared during the i v glucose tolerance test, varying from 0 to 2 g/hr usually below 0 5 g It is suggested that the tests used provide excellent means for the differentiation between simple hyperthyroid cases with possible alterations in glucose metabolism and cases with hyperthyroidism and diabetes mellitus

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Acute Renal Failure Due to Carbon Tetrachloride Poisoning

By

VIGGO KAMP NIELSEN and JENS LARSEN

Although carbon tetrachloride (CCl_4) has been abolished as an anthelminthic (9) in most parts of the world, poisoning with this agent, not infrequently with a fatal outcome, still occurs due to its widespread use as a solvent in industry as a household remedy for dry cleaning, and as a fire extinguisher

CCl_4 is readily absorbed through the lungs and more slowly from the gastrointestinal tract this latter absorption being considerably increased however, by concomitant ingestion of fat and alcohol (22 26) It is found in high concentrations in fatty tissues liver brain bone marrow and kidneys During the first six hours after ingestion 50—60 % is exhaled through the lungs a minor part is excreted through the stools and urine in partly decomposed form (19) whereas the rest is eliminated very slowly being demonstrable in the expiratory air for several weeks (30)

Inhalation is by far the most common way of intoxication today, the relatively few cases of oral poisoning recorded in recent years being due to mistakes or attempts at suicide (8, 20) The clinical picture is roughly similar except for a tendency towards more severe hepatic lesions following oral ingestion Few hours following exposure the patient may experience cerebral symptoms, dizziness, headache blurred vision, fatigue later followed by gastrointestinal disorders, nausea, vomiting, and abdominal pain Respiration may become laboured and pulmonary edema often complicated by pneumonia can ensue A few days later a transitory jaundice develops often accompanied by tender enlargement of the liver, epistaxis or other hemorrhagic manifestations The frequency of renal impairment was first described by Franco (7) Oliguria, albuminuria and microscopic hematuria begin varying from one to eight days after exposure and in severe cases acute renal failure may ensue A minor group develop a toxic myocarditis (16)

Pathological lesions are almost constantly found in liver and kidney They

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consist of centrilobular necroses in the liver and degeneration of the tubular epithelium in the kidney (23, 29, 36).

In the treatment of acute poisoning by CCl_4 the artificial kidney has gained an increasing importance during recent years. In contrast to "dialysable poisons" (15, 27, 28) hemodialysis does not aim at an elimination of the poison, but is directed towards the acute uremic syndrome and follows the generally accepted indications for dialysis in acute renal failure.

This report presents five cases of acute CCl_4 intoxication with renal functional impairment treated at the dialysis center at Rigshospitalet since 1958. The pitfalls in the differential diagnosis are described, and an evaluation of the more favourable prognosis following active treatment of the acute renal failure is given based on our own results and on reports from the last decade.

Case reports

Case 1. A 28 year old man was admitted to the dialysis center at Rigshospitalet on Sept. 25th, 1958, because of acute oliguria 8 days after attempting suicide by inhalation of carbon tetrachloride.

Past history. No information about previous illness or excessive alcohol consumption.

Present illness. On Sept. 17th, 1958 during an exogenous depression the patient inhaled carbon tetrachloride from a moistened cotton tampon in a plastic bag, which he had pulled over his head. The exact duration of exposure is unknown, but after a while he pulled off the bag and went to sleep in his car with the bag beside his head. Having slept for approximately 24 hours he woke up with heavy headache and low back pain later followed by diffuse abdominal pain, anorexia and facial edema. He returned to his home, from where he was admitted to hospital on the fourth day after exposure to CCl_4 because of oliguria, proteinuria and hematuria. On the ninth day he was transferred to the dialysis center because of continued oliguria and azotemia under the diagnosis of acute renal failure due to carbon tetrachloride poisoning.

Physical examination. On transfer the patient was found in good general condition, normohydrated but slightly jaundiced. Stethoscopy of heart and lungs was normal. The abdomen was soft without tenderness or hepatomegaly. Temperature was 36.6° C, pulse rate 66/min. and B. P. 170/100 mm Hg. A slight proteinuria was found. Urine microscopy, chest X-ray, and ECG was normal. Relevant laboratory findings concerning liver and kidney functions appear from fig. 1.

Course and treatment. From the day of transfer the patient was treated with conventional conservative regimen supplemented by hemodialysis on the 10th day. The 24 hours endogenous creatinine clearance remained below 5 ml/min. for 16 days (fig. 1). The proteinuria persisted until the 24th day after exposure. The patient was discharged on the 35th day with a serum urea of 46 mg/100 ml, normal serum electrolytes and a 24 hours endogenous creatinine clearance of 70 ml/min. Urine output was 5.2 l/24 hours with a specific gravity of 1.008.

Mild liver damage was demonstrated by elevation of the SGPT, elevation of serum bilirubin to a maximum of 2.0 mg/100 ml and by slightly reduced prothrombin-proconvertin values. These abnormalities were quite transitory (fig. 1).

At control admission three months later the patient was found in good health. Urine output varied between 580 and 1280 ml/24 hours with a specific gravity of 1.010—1.026. The urine did not contain protein or blood but a mild leucocyturia and coliuria was demonstrated. 24 hours endogenous creatinine clearance was 115 ml/min. Serum urea, serum electrolytes and liver function tests were normal.

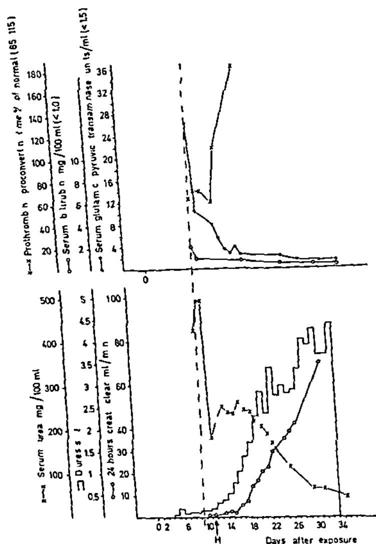


Fig 1 Clinical course of case 1 H hemodialysis Stippled line indicates day of transfer

At control 11 months later the coluria had disappeared and liver and kidney function remained normal (We have later been informed that the patient succeeded in committing suicide on March 26th 1960)

Case 2 A 33-year-old man was admitted to the dialysis center at Rigshospitalet on Jan 29th 1962 because of acute renal failure 5 days after having been exposed to vapours of carbon tetrachloride during his work

Past history At the age of 28 the patient was admitted with monosymptomatic hematuria Urography was normal and there were no further urological symptoms Otherwise he had formerly been healthy Alcohol consumption was stated to be moderate

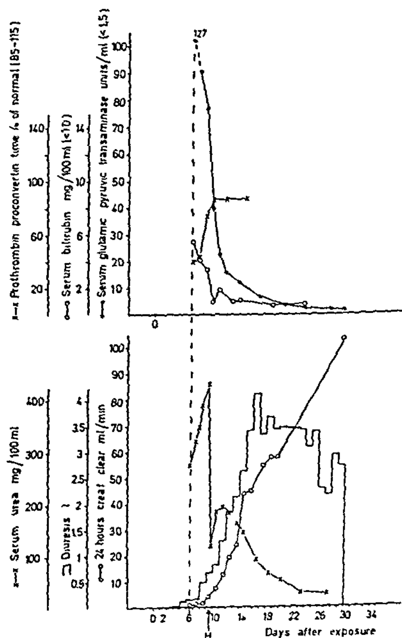


Fig 2 Clinical course of case 2 H = hemodialysis Stippled line indicates day of transfer

Present illness On Jan 24th, 1962, the patient was cleaning a relay by means of a sprayer with carbon tetrachloride There was no history of alcohol consumption while he performed this job The patient experienced transitory nausea and vomiting During the following two days he suffered from intermittent nausea, vomiting and periumbilical pain Acute appendicitis was suspected and the patient was admitted to a surgical department on the third day after exposure At that time he presented jaundice oliguria, proteinuria and a rising serum creatinine. Subsequently he was transferred to the medical department of the same hospital but as oliguria persisted he was admitted to the dialysis center on the sixth day after exposure under the diagnosis of acute renal failure of unknown origin since the history of exposure to CCl_4 had been completely overlooked

Physical examination On transfer the patient complained of nausea, accompanied by vomiting and hiccups He was mildly jaundiced and had moderate epigastric tenderness without enlargement of the liver Stethoscopy of heart and lungs was normal Temperature was 37 4 C, pulse rate 64/min and B P 145/95 mm Hg The urine contained 0 1 g protein per 24 hours Microscopy chest X ray and ECG were normal Liver and kidney function is demonstrated in *fig 2*

Course and treatment From the day of transfer the patient was treated by conventional measures supplemented by hemodialysis on the ninth day (fig 2) The 24 hours endogenous creatinine clearance remained below 5 ml/min for 11 days and the proteinuria persisted until the 25th day At discharge on the 29th day serum urea was 26 mg/100 ml *serum* electrolytes were normal and 24 hours creatinine clearance 100 ml/min Urine output was 2 7 l/24 hours with a specific gravity of 1 005

The *hepatotoxic* effect was demonstrated by jaundice abnormal liver function tests (fig 2) and by a liver biopsy taken on the 15th day after exposure The hepatic tissue presented several small centrilobular necroses The liver function tests soon however became normal

At control four weeks later the patient complained of headache and was easily exhausted Seven weeks later he felt quite well and could return to work. On both controls liver and kidney function tests were found to be entirely normal

Case 3 A 58-year-old diver was admitted to the dialysis center at Rigshospitalet on June 14th 1962 because of acute renal failure 4 days after having consumed 10—15 ml carbon tetrachloride by mistake

Past history At the age of 21 the patient had jaundice of unknown etiology for 2 weeks and at the age of 31 he passed a renal calculus The patient admitted to have had a moderate but steady alcohol consumption for the last few years prior to the present illness

Present illness On *June 10th, 1962, the* patient had a few drinks of Danish aquavit with a friend Following this alcohol consumption *he happened to serve another drink from* a bottle containing carbon tetrachloride Although he immediately *realized* the mistake *he could not help* swallowing 10 to 15 ml of the CCl_4 After 7 *hours* at ease he suddenly felt ill with profuse perspiration, nausea vomiting and violent *abdominal pain* This *continued* on the following day where the urine became dark and sparse On the third day *the practi*tioner noticed jaundice and the patient was admitted to hospital from where *he* was transferred to the dialysis center on the fourth day under the diagnosis of acute oliguric renal failure due to CCl_4 poisoning

Physical examination At transfer the patient was found intensely jaundiced He was complaining of nausea and moderate abdominal pain Stethoscopy of the lungs was normal but auscultation of the heart revealed a pericardial friction rub of varying intensity The liver was tender and enlarged reaching 4 cm below the right costal margin The temperature was 36 8 C pulse rate 64/min and B P 175/95 The urine contained 3 3 °/₀ protein microscopy revealed a few leucocytes and 10—25 erythrocytes Serum electrophoresis showed a slight hypoalbuminaemia (3 85 g/100 ml) whereas the other fractions were normal ECG was normal Other laboratory findings are shown in fig 3

Course and treatment The acute renal failure was treated by conventional measures including one hemodialysis on the 10th day (fig 3) 24 hours endogenous *creatinine* clearance remained below 5 ml/min for 12 days and proteinuria persisted for 27 days A coliuria was treated with *streptoduocin* At discharge on the 60th day creatinine clearance was 75 ml/min but a mild polyuria and a low specific gravity *persisted Serum* electrolytes were normal

As shown in fig 3 this patient presented severe hepatotoxic lesions A hypoprothrombinaemia was normalized in five days following administration of water soluble vitamin

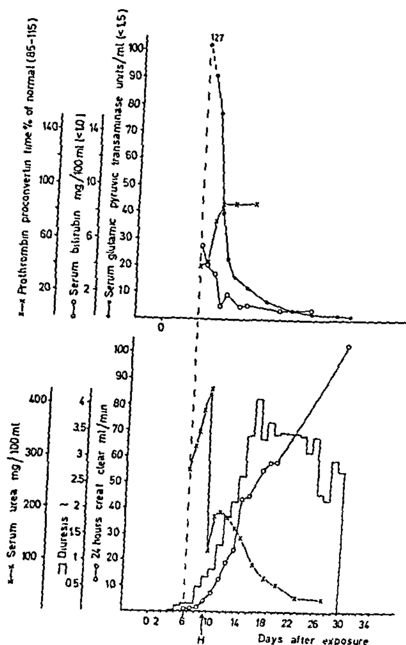


Fig 2 Clinical course of case 2 H = hemodialysis Stippled line indicates day of transfer

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fibrilloflutter Digitalis was administered with gradual effect on the heart failure On discharge the auricular fibrillation was still present, but T waves were normal

On readmission 3 months later the patient complained of fatigue and functional dyspnoea No edema was present. Auricular fibrillation was still present but sinus rhythm was established on quinidine treatment Six months later treatment with quinidine was discontinued Sinus rhythm was maintained and the patient was able to return to work

At controls 1/2—1 and 2 years later liver and renal functions were found quite normal whereas the heart remained moderately enlarged

Case 4 A 40-year-old man was admitted to the dialysis center at Rigshospitalet on June 7th 1964 because of acute renal failure 10 days after having been exposed to CCl_4 during his work

Past history At the age of 21 the patient was in hospital with hepatitis In the past 15 years he had a chronic abuse of alcohol

Present illness On May 29th the patient was cleaning metal pieces in a bath containing carbon tetrachloride During work which took place in a small unventilated room considerable amounts of alcohol were consumed On the following days the patient complained of headache abdominal pain and nausea later followed by vomiting and diarrhoea Temperature rose to a maximum of 39 8 C, and on the third day the patient noted a small urinary output Because of heavy polypnoea moderate jaundice and slight tenderness below the right costal margin the patient was admitted to hospital on the sixth day under the tentative diagnosis of pneumonia and alcoholic cirrhosis since the exposure to CCl_4 had been completely overlooked As the patient presented progressive oliguria azotemia and increasing hyperkalemia he was transferred to the dialysis center on the 10th day after exposure

Physical examination The patient was found sweating polypnoeic and somnolent Stethoscopy revealed reduced ventilation of the basal parts of the lungs with moist rales and a distinct pericardial friction rub The liver was tender and reached 5 cm below the costal margin There was no jaundice, spider angiomata or palmar erythema Temperature was 38 2° C, pulse rate 120, and B P 150/105 The urine contained 0 6 g protein per 24 hours and microscopy showed numerous erythrocytes 8—12 leucocytes and 2—3 epithelial cells. Chest X ray showed extensive perihilar infiltration and moderate pulmonary congestion ECG was normal Other laboratory findings are shown in fig 4

Course and treatment The patient was treated with conventional measures including two hemodialyses (fig 4) 24 hours endogenous creatinine clearance remained below 5 ml/min for 19 days and proteinuria persisted for 20 days He went through a protracted polyuric phase with considerable saltloss demanding careful supervision of the electrolyte balance A renal biopsy was taken on the 30th day showing flattened tubular epithelium and hemoglobin casts without pathological changes in the glomeruli and vessels At discharge renal functions were normal except for the concentration power The urine was sterile

The slight hepatic damage was transitory and liver function tests were soon normal (fig 4) A hepatic biopsy on the 32nd day was normal

The pericardial friction rub soon disappeared and X ray did not show any sign of exudative pericarditis Numerous ECGs however showed the presence of an asymptomatic toxic myocarditis with depression of the T waves in lead I and II which persisted for several weeks

On admission for control 4 months later the patient was found normal regarding renal liver and cardiovascular functions

Case 5 As this patient a 38-year-old man had been occupied by the same work as the former one he was suspected to suffer from CCl_4 intoxication and at our request he was taken into hospital for further examination As laboratory findings revealed azotemia serum urea being 217 mg/100 ml and serum

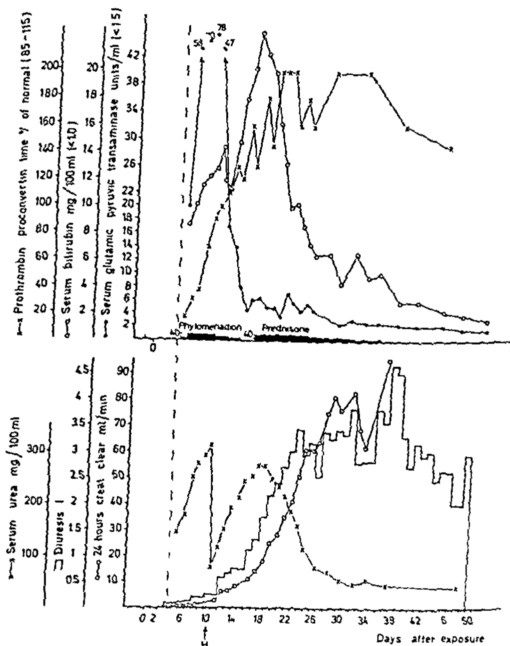


Fig. 3. Clinical course of case 3. *H* = hemodialysis. Stippled line indicates day of transfer

K. Serum bilirubin rose to a maximum of 22.9 *mg/100 ml on* the 14th day only interrupted by a moderate fall following dialysis. Pronounced jaundice and marked tender enlargement of the liver persisted for 39 days. On the 16th day administration of prednisone was instituted, while additional signs of parenchymatous lesions of the heart were encountered.

During the dialysis auricular fibrillation commenced accompanied by elevation of the B.P. (max. 225/120). B.P. remained elevated for the following 8 days, pulse rate was accelerated, periodically arrhythmic, and the patient had moderate dyspnoea. Chest X-ray revealed increased heart size and slightly congested lungs. The ECG showed negative T waves in lead II and III and auricular

noted moderate jaundice and urine output became sparse and dark Temperature rose to a maximum of 39 6 C. Pneumonia was suspected and he was given penicillin following which the temperature fell

On transfer, *physical examination* showed a fairly good general condition of the patient. Temperature pulse rate, and B P were normal Urine output was 3730 ml/24 hours and the urine contained neither protein nor pathological elements Serum urea was 245 mg/100 ml serum creatinine 22 2 mg/100 ml 24 hours endogenous creatinine clearance 8 ml/min Serum electrolytes were normal Prothrombin proconvertin time was 80 % of normal serum bilirubin 1 5 mg/100 ml SGOT 2 1 units/ml (normal value $< 1\ 7$ units ml) SGPT 6 8 units/ml (normal value 1 5 units ml) LDH 36 units/ml (normal value 23 units ml)

Chest X ray showed moderately dense structure in the right basal lobe ECG was normal

Course and treatment The clinical course was uncomplicated This patient demonstrated the typical picture of the polyuric phase of acute renal failure Treatment consisted of careful supervision of the electrolyte balance and replacement of water and electrolyte loss No hemodialysis was needed and creatinine clearance became normal in 8 days

Although liver function tests showed only very moderate signs of hepatotoxic lesions a hepatic biopsy on the 29th day after exposure revealed marked centrilobular necrosis Liver function as well as renal function was normal at discharge

Discussion

The case histories in this paper all show typical variations of the CCl_4 intoxication with regard to exposure as well as to the clinical course They clearly demonstrate the diagnostic difficulties and the prognostic value of hemodialytic treatment

Diagnosis It is of primary importance that a correct diagnosis is made as soon as possible but this may be far from simple The diagnosis is easy if ingestion by mistake arouses the suspicion of the patient, or if attempt at suicide is committed with a chemical available for analysis These situations are exemplified by case 1 and 3, where diagnosis was made before transfer to our department

In the majority of cases however, intoxication follows unrecognized inhalation of vapours, and only a careful inquiry into the possible exposure to CCl_4 will reveal the relationship It may however, be a difficult task since the offending situation is often far back in the memory of the patient due to the free interval of variable duration between exposure and initial symptoms of intoxication Therefore the clinical picture is commonly misinterpreted as some gastrointestinal infection hepatitis, cholecystitis chronic alcoholism or pneumonia Not infrequently the patients are admitted to surgical departments under the diagnosis of acute abdomen and some patients have been appendectomized because of suspected appendicitis Oliguria is a late symptom and is often overlooked or misinterpreted as a result of dehydration due to persistent vomiting Our cases nos 2 4 and 5, where the correct diagnosis was *not made before* transfer, exemplifies this course of events

It appears reasonable to conclude that any patient who presents a clinical picture of gastrointestinal hepatic and renal symptoms should be suspected of suffering from CCl_4 intoxication Careful inquiry into the immediate past history with respect to this possibility is

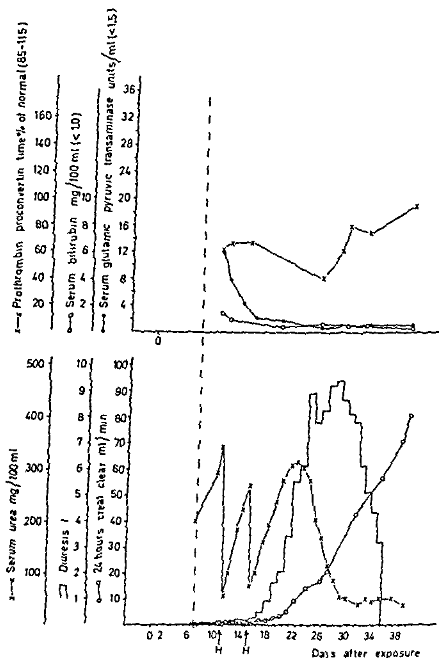


Fig 1 Clinical course of case 4 H – hemodialysis Stippled line indicates day of transfer

creatinine 15 mg/100 ml he was immediately transferred to the dialysis center at Rigs hospitalet on the 15th day after exposure (June 12th, 1964)

His *past history* revealed nothing of special interest apart from a chronic abuse of alcohol for many years

He had been less heavily exposed to CCl_4 than his companion as he had been working outdoors part of the time but he had joined him in heavy alcohol consumption In the following days he became extremely ill with laboured respiration coughing vomiting nausea and abdominal pain Later his family

1954 On the basis of our favourable results and the figures in table I we find it justifiable to conclude, that all cases of CCl_4 poisoning with impaired renal function should be transferred as early as possible to a center for dialysis treatment By these means, undoubtedly, the prognosis could be further improved

Despite the changed prognosis of severe toxic lesions due to modern treatment, it must be strongly emphasized, however that CCl_4 is still a most dangerous poison which constitutes an important public health hazard Regulations on warning labelling of CCl_4 products and improved industrial hygiene play an important role in the prevention of intoxication, but it should be stressed too that in most uses CCl_4 may readily be replaced by less toxic chemicals

Summary

Five cases of acute renal failure following acute intoxication with CCl_4 have been treated since 1958 Detailed case reports are given Four patients developed protracted oliguria, necessitating treatment with the artificial kidney Renal function was restored to normal in all patients The diagnostic difficulties are commented and a careful inquiry into the possible exposure to CCl_4 is advised in every case presenting concomitant gastrointestinal hepatic and renal symptoms of uncertain origin

The value of active treatment, including dialysis in cases of CCl_4 poisoning with renal failure is illustrated by our own results as well as by a review of the literature

Among 128 cases, collected from the literature of the last decade, 120 presented renal failure, 44 were dialysed, and the overall mortality was 17 per cent Among 77 cases, published between 1939 and 1953 (i e from a period during which hemodialytic treatment of uremia had not yet gained widespread usage), 74 presented renal failure, and the overall mortality was *36 per cent*

Despite the improved prognosis following the introduction of hemodialytic management of renal failure due to CCl_4 poisoning, this intoxication is, however, still an important public health hazard For most purposes CCl_4 could readily be replaced by other, less toxic, chemicals

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TABLE I Three consecutive materials of intoxication by CCl_4

Material (collected by)	Total no	Renal involvement (% of total)	Fatal cases (% of total)
Up until 1939 (Smetana 1939)	141	33 (24%)	39 (28%)
1939—1953 (Hardin 1954)	77	74 (96%)	22 (36%)
1953—1965 (Kamp Nielsen & Larsen)	128	120 (94%)	22 (17%)

therefore indicated in all such cases A further aid in the diagnosis has recently been developed by Stewart (30) Stewart was able to demonstrate CCl_4 in blood and expired air of an intoxicated patient using spectrophotometric analysis in infra red light (31) By serial analyses he was able to draw the elimination curve and calculate the magnitude of exposure The compound was demonstrable several weeks after exposure This seems to be an important diagnostic tool, as the method is stated to be specific, sensitive and simple

Prognosis A review of the literature clearly points to the dramatically changed prognosis following the invention of dialysis as the most efficient supplement to conventional treatment of acute renal failure

Two major materials deserve special mention (table I) In 1939 Smetana (29) presented the first statistical evidence of the importance of the nephrotoxic action of CCl_4 He collected from literature 141 cases of acute CCl_4 poisoning Symptoms of renal damage were present in 33 cases (or 24 %) and 39 cases (28 %) were fatal

In 1954 Hardin (10) supplemented this material by 77 cases reported between 1939 and 1953 Renal involvement was present in 96 % and 28 cases (36 %) were fatal

In order to evaluate the therapeutic and prognostic importance of hemodialysis in CCl_4 intoxication, we have collected 128 cases of acute intoxication by CCl_4 from the literature since 1953 (cf table I and ref 1—6, 8, 11—14, 17—18, 20—21, 24, 25, 31—35) Acute renal failure developed in 120 cases (94 %) and dialysis was performed in 44 cases (37 % of cases with renal failure) Twenty two patients died (17 % of total) but it should be noted, that at least 13 patients (10 % of total) died from acute hepatic insufficiency Eight of the 22 patients had been dialysed

The frequency of renal involvement is considerably higher in Hardin's and in our own material than in that of Smetana This may be due to lack of recognition of renal symptoms before 1939 or perhaps to the fact that intoxication by ingestion has become rare compared with that of inhalation

There is good agreement between the frequencies of renal impairment in Hardin's and our materials In contrast the mortality has been greatly reduced It seems justifiable to contribute this reduction to the hemodialytic treatment which first came into wide scale use after

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Pine Pollen Pneumoconiosis in the Rat, and its Relation to Sarcoidosis

By

CARL JOHAN GOTHE

As early as 1940 it was observed that the frequency of sarcoidosis is highest in the countryside (32) This observation was confirmed during the early 1950s in connection with comprehensive epidemiological studies of this disease in the armed forces of the U S A (4 11, 21) These investigations revealed also other characteristic features in the geographical distribution of sarcoidosis which were used, among other applications, as a clue in the attempts to ascertain the etiology of the disease In view of the fact that the frequency of sarcoidosis in the U S A seems to run parallel with the occurrence of coniferous forests (6, 7 10) an etiological connection between exposure to pine pollen and sarcoidosis has been suspected (6 7) A certain — but not always regular — correspondence between the geographical diffusion of sarcoidosis and coniferous forests has also been reported from Sweden (31) South Africa (29) Israel (25) and certain states in the U S A such as Florida (18) and Louisiana (26) On the other hand in countries such as Denmark (1), Switzerland (27), Scotland (9), Japan (15), and Uruguay (24) it has not been possible to observe any such correspondence Sarcoidosis has been found to occur also in countries such as Hungary (19) and Egypt (12), where there are no pine forests

In support of the hypothesis that there exists an etiological connection between exposure to pine pollen and sarcoidosis, it has been stated that pine pollen displays acid fast staining properties which are similar to (8), but not identical with (30) those of the tubercle bacilli which can give rise to tissue changes that closely resemble those found in sarcoidosis Biochemical (8) and immunological (28) similarities between pine pollen and tubercle bacilli have also been described Moreover a number of experiments have been reported which show that pine pollen can cause epithelioid cell granulomas in experimental animals (17 30) These observations have been called in question by others who consider that

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and the numerical estimation of the macroscopically and microscopically demonstrable tissue reac

Lung weight of animal / Body weight (°)	Fluid content of lungs (%)	Hydroxyproline level of lungs (mg/g dry weight)	Degree of morphological pulmonary changes	
			Macro	Micro
0 8[illegible]±0 08	78 6±2 0	11 6±1 5	1 9	2 8
0 58±0 04	79 4±0 6	13 4±0 9	0 3	0 8
0 80±0 09	79 2±0 6	14 4±1 7	1 9	3 0
0 [illegible]6±0 0[illegible]	80 1±0 5	*13 1±0 8*	*0 5*	0 0
0 72±0 07	78 4−1 5	13 3±1 3	1 5	2.4
0 62±0 07	78 2±1 6	12 1±1 2	0 7	1 0
			1 4	2 2
			0 5	0 0

because all the animals in this group manifested signs of unspecific infection in the respiratory organs

an analytical balance during the actual autopsy The hilar and paratracheal lymph nodes and one lung (alternate right and left) from each animal were then fixed in formalin for further histological preparation The other lung was dried in a thermostat, and then the dry weight and hydroxyproline content of the organ were determined

Large atelectases pronounced emphysema and macroscopically demonstrable bronchiectases with mucopurulent contents were found to cause a considerable increase both in the lung weight and in its hydroxyproline content (absolutely and relatively) Since such changes occurred in individual animals in both the pine pollen groups and the control groups they were assumed to be caused by unspecific infections This was supported also by the nature of the histological changes associated with them Consequently organs exhibiting such changes were excluded from the statistical analysis of the organ weights and of the values obtained for their hydroxyproline content In view of the fact that the histological character of these changes differs markedly from the specific tissue reactions induced by pine pollen it has in general been possible however to include organs with such changes in the histological appraisal of the tissue reactions induced by pine pollen

The macroscopic tissue reactions were graduated according to a 6-point scale where point 0 indicates the quite normal lung structure and point 5 indicates intense fibrosis of the type that occurs in fibrotic pneumoconiosis, e g silicosis.

The microscopically demonstrable tissue reactions in the lungs were graded on a 7-point scale where point 0" also indicates the normal lung structure whereas point 6 indicates fibrosis of the type which occurs in advanced fibrotic pneumoconiosis The graduation was based on the cellular reaction assessed by means of tissue sections stained with hematoxylin-eosin and on the fibrotic changes assessed by means of sections Ag-impregnated according to Gomori's method The deposition of pine pollen fragments in the pulmonary parenchyma was studied in sections stained with carbol fuchsin according to Ziehl Neelsen's method and by the periodic acid Schiff reaction

The hydroxyproline contents of the lungs were determined by a modification of the method introduced by Neuman and Logan (23)

TABLE I Animal and organ weights, the relative fluid and hydroxyproline contents of the lungs, tions in the lungs of the different animal groups Mean±S D

Months	Animal group	No of animals at the end of the experiment	Weight of animals (g) Start	End	Hilar and paratracheal nodes Wet weight (mg)	Lungs Wet weight (mg)
1	Exp	8	213	242	67±16	2 035±191
	Control	4	208	238	23± 7	1,376± 86
2	Exp	8	211	253	56±23	2,014±224
	Control	4	213	254	17± 2	1,408±108
4	Exp	8	221	282	43±14	2,056±135
	Control	3	205	263	23±17	1,630±170
6	Exp [1]	5	215	275		
	Control[1]	2	218	295		

[1] Data on organ weights and the relative fluid and hydroxyproline contents have not been given,

the pine pollen reaction is an example of an unspecific 'foreign-body" reaction (13), or that it is of an irritative and inflammatory nature, but without giving rise to real epithelioid cell granulomas or giant cells of a 'foreign body" type (2)

Thus, the studies cited on the possible connection between pine pollen and sarcoidosis have yielded contradictory results This has even caused the author of the 'pine pollen theory' M M Cummings, to review his hypothesis (5) However, at the present stage it appears premature to reject any possibility of a connection between these two factors and, consequently, it has been considered expedient to attempt to shed more light on this problem

Material and methods

Female rats (Sprague Dawley) were used as experimental animals, which, at the beginning of the experiment, had a body weight of 200—220 g Under light ether narcosis 20 mg of pine pollen (Pinus sylvestris), suspended in 1 ml of physiological saline, were injected intratracheally into each experimental animal Since the natural size of pine pollen grains (ca 30×40×70 microns) can jeopardize an even distribution and quantitative deposition of the particles in the pulmonary parenchyma the pollen grains were ground to form particles 1—3 microns in size before the suspension was prepared

The albino rats treated with pine pollen were divided into 4 groups of 8 animals, which were killed after 1, 2 4 and 6 months The control animals (4 rats per group treated) were injected intratracheally with 1 ml of physiological saline The animals treated with pine pollen and the corresponding controls were kept together in order to avoid the sources of error which can occur as a result of different conditions of management

At autopsy an assessment was made of the macroscopically demonstrable changes in the lungs hilar lymph nodes and paratracheal lymph nodes The wet weights of these organs were determined by weighing them on



Fig 4 Lung of rat killed 1 month after intratracheal injection of 20 mg of ground pine pollen (*Pinus sylvestris*) suspended in 1 ml of physiological saline (Ag impregnation according to Gomori × 67)

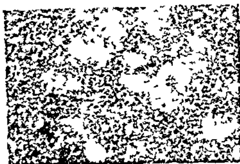


Fig 5 Hilar lymp node of rat killed 1 month after intratracheal injection of 20 mg of ground pine pollen (*Pinus sylvestris*) suspended in 1 ml of physiological saline (Htx-eosin × 67)

hematoxylin eosin, it was as a rule, distinguishable in the Ag impregnated sections The fibrils of the connective tissue were thin and formed a loose network within the clusters of epithelioid cells

The pulmonary tissue reaction was essentially the same 2 4 and 6 months after the intratracheal administration of the pine pollen (fig 3) The epithelioid cell granulomas however appeared to gradually decrease in size and became more and more distinctly demarcated from the normal lung tissue

The hilar and paratracheal lymph nodes contained as a rule an abundance of rounded foci of epithelioid cell like cells with palely staining cytoplasm (fig 5) However no giant cells could be shown to be present Pollen fragments occurred in these foci but not in the areas with normal lymph node structure This lymph node reaction was most pronounced 1—2 months after the intra tracheal injection of pine pollen and subsequently there was a marked re gression

No tissue reactions of the types de scribed were observed in the organs of the control animals

Discussion

Pine pollen deposited intrapulmonarily causes a pronounced tissue reaction, which is reflected both in an increased weight of the lungs and their regional lymph nodes and in a macroscopically and microscopically demonstrable change in the lung and lymph node structure

The values shown in table I indicate that pine pollen pneumoconiosis in rats reaches its peak 1—2 months after the intratracheal injection of pine pollen and then successively decreases No significant change in the relative hydroxypro line content of the lungs is observed Since hydroxyproline can be regarded as a biochemical index of collagen (23), this indicates that pine pollen pneumo coniosis in rats has little fibrotic tendency Nor can the increased lung weights due to the pine pollen reaction be explained

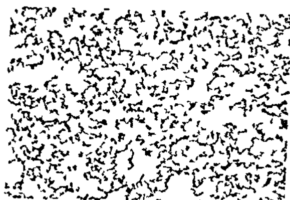


Fig 1 Lung of rat killed 2 months after intratracheal injection of 1 ml of physiological saline (Htx eosin × 67)



Fig 3 Lung of rat killed 4 months after intratracheal injection of 20 mg of ground pine pollen (*Pinus sylvestris*) suspended in 1 ml of physiological saline (Htx eosin × 67)



Fig 2 Lung of rat killed 1 month after intratracheal injection of 20 mg of ground pine pollen (*Pinus sylvestris*) suspended in 1 ml of physiological saline (Htx eosin × 67)

Results

Table I shows the mean values for the animal and organ weights, and the relative fluid and hydroxyproline contents of the lungs in the different animal groups The table also gives the mean values in the different animal groups of the numerical estimation of the macroscopically and microscopically demonstrable tissue reactions in the pulmonary tissue

The character of the histological changes is shown in figs 1—5

One month after the intratracheal injection of the pine pollen suspension (figs 2 and 4), in all the animals investigated there was a pronounced and uniform tissue reaction in the lungs, with an abundant occurrence of epithelioid cell clusters In these there was often found an admixture of lymphocytic elements In part these formations were rather diffusely demarcated from the surrounding lung parenchyma, and in part they were more distinctly demarcated and appeared as granuloma-like cell conglomerations There was an abundance of giant cells which, as a rule, were of the Langhans' type with the nuclei situated concentrically in the cell peripheries Giant cells of a foreign body ' type with the nuclei situated more centrally were also observed There was no necrosis

On PAS staining and carbol fuchsin staining by the Ziehl Neelsen method an abundance of pine pollen fragments was manifested in the granulomatously changed areas they were especially numerous in the cytoplasm of the giant cells

There was a tendency to fibrosis within the granulomatously changed areas (fig 4), but despite the fact that the alveolar structure in places was almost entirely obliterated in the sections stained with



Fig 4 Lung of rat killed 1 month after intratracheal injection of 20 mg of ground pine pollen (Pinus sylvestris) suspended in 1 ml of physiological saline (Ag impregnation according to Gomori × 67)



Fig 5 Hilar lymp node of rat killed 1 month after intratracheal injection of 20 mg of ground pine pollen (Pinus sylvestris) suspended in 1 ml of physiological saline (Htx-eosin × 67)

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by tissue edema, since no significant differences are observed between the animals treated with pine pollen and the corresponding controls, with regard to the fluid contents of the lungs. On the other hand, the established changes in the lung weights may be accounted for by a mainly cellular reaction which is maximal 1—2 months after the administration of the pine pollen, and thereafter gradually diminishes. This is also in good agreement with the histological findings.

The general condition of the animals treated with pine pollen was quite unaffected during the entire experimental period. This subjective impression is supported by the fact that the increase in the body weights of both the experimental and the control animals ran parallel throughout the period in question.

Pine pollen pneumoconiosis in the rat displays many morphological and clinical similarities with human sarcoidosis. Both diseases are, in the main, of a reversible nature, despite the fact that the tissue changes may persist for a long time. They are characterized by epithelioid-cell granulomas in which giant cells of the Langhans' type are often present. Both in sarcoidosis and in pine-pollen pneumoconiosis the granulomas are diffusely distributed equally in both lungs and in both diseases there is usually a lymph-node reaction which is especially pronounced in the hilar and paratracheal nodes. One difference is that the granulomas of pine pollen pneumoconiosis in the rat appear to have a more marked admixture of lymphocytic cells than is usually found in the granulomas of human sarcoidosis. Whether this difference is dependent upon dissimilarities in the etiology and pathogenesis of these two diseases, or upon species differences between rats and human beings, cannot be determined from this investigation.

In view of the results of the animal experiments reported here, it may be expected that, if the disease "pine pollen pneumoconiosis" occurs in human beings, it will exhibit both clinical and morphological similarities with sarcoidosis. The peculiarities, previously described, with regard to the geographical diffusion of sarcoidosis, also led to the supposition of a positive connection between pine-pollen exposure and this disease. Nevertheless it is improbable, especially for the following reasons, that sarcoidosis is an example of pine pollen pneumoconiosis, except perhaps exceptionally.

a) Pine pollen grains, like pollen grains of other coniferous species, are much too large (about 30×40×70 microns) to be able to penetrate through the bronchioli into the alveoli in such quantities as to give rise to a diffuse and significant dissemination of epithelioid cell granulomas in the pulmonary parenchyma and the hilar lymph nodes. Inhaled particles the diameter of which exceeds 5—10 microns are very effectively filtered off in the upper respiratory tract (3, 14), and particles larger than 3 microns are of little significance in dust exposures (14). The pollen grains of conifers are highly resistant to mechanical damage, desiccation and other physical influences. This fact has been utilized e. g. in paleontology and archeology, since it is possible to find an abundance of morphologically classifiable pollen grains in old geological formations. Consequently the risk of finely particulated pine pollen fragments occurring naturally

in significant quantities is *probably extremely small*

b) With the Ziehl Neelsen method for staining tissue sections which display the characteristic changes of sarcoidosis "acid fast" material usually cannot be observed. At times however, "acid fast" bacilli (mycobacteria) are found in the granulomatous tissue (33) which is probably a manifestation of the old observation that tuberculosis can occur *in* morphological types which are difficult to distinguish from those in sarcoidosis. On the other hand it is not possible to find pollen grains or pollen fragments in the granulomatous tissue. If *inhalation* of pine pollen were a common cause of sarcoidosis then such findings should also be common as is the case in pine pollen pneumoconiosis in the rat. The nature of the inclusion bodies (Schaumann bodies and asteroid bodies) which are sometimes found in sarcoidosis is unknown. Their morphology and stainability make it unlikely however, that they originate from pollen grains of the pine or other gymnosperms.

c) Sarcoidosis occurs also in areas which are devoid of coniferous forest vegetation although the frequency of sarcoidosis appears to be especially high in areas with a temperate climate where conifers often are dominant elements in the forests.

Consequently if there is a positive connection between pine pollen exposure and sarcoidosis, it is not probable that the factor which induces sarcoidosis is the pollen grains as such. On the other hand it is possible that this factor can be disseminated by pollen from conifers e g by adhesion to the surfaces of the pollen grains from which it may be liberated in the respiratory tract and then penetrate into the pulmonary parenchyma. *If the pollen grains — perhaps* together with finely particulated dust from other plants such as peanuts (16) — *serves* only as an optional vector for the etiological factor, this may explain the fact that there can be a high frequency of sarcoidosis in areas rich in coniferous forests, and that, at the same time, the disease can occur also *in* areas without such forests. The nature of this hypothetical agent that provokes sarcoidosis is obscure. Investigations now in progress show, however, that colonies of fungi of various types can almost regularly be cultivated from newly collected pine pollen (20). The relationships between the fungi and the pollen grains are probably of great biological importance and the examination *of the fungal relations* of pollen grains is a new field of research, and may be deemed to be of importance in considerations of pollen viability and the dissemination *of parasitic* fungi (22).

Summary

Female albino rats were injected intratracheally with 20 mg of ground pine *pollen (Pinus sylvestris) suspended in 1* ml of physiological saline. The animals were killed 1, 2, 4 and 6 months after the administration of the pine pollen. The organ *weights and the hydroxyproline* contents of the lungs were *determined* and the tissue reactions in the lungs, hilar and paratracheal lymph nodes were studied. These reactions were characterized by granulomatous changes without significant fibrosis in which an abundance of Langhans giant cells and some foreign body giant cells were *observed* on the other hand there was no necrosis. The tissue reaction culminated after 1—2 *months* and then gradually

decreased The general condition of the experimental animals was unaffected Thus, pine pollen pneumoconiosis in rats displays both morphological and clinical similarities with human sarcoidosis

If a positive connection exists between pine pollen exposure and sarcoidosis, it is improbable, for reasons discussed, that the etiological factor is the pine pollen as such On the other hand, it is possible that this factor can be disseminated together with the pollen grains, e g through adhesion to their surfaces The nature of this hypothetical agent is obscure However, the relation between pollen and fungi is emphasized

Acknowledgement

This investigation was supported by grants from the Swedish National Association against Heart and Chest Diseases

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From Medical Department A University of Bergen School of Medicine (Head O J Broch M D), Bergen Norway and Nuffield Department of Clinical Medicine (Head L J Witts) The Radcliffe Infirmary Oxford England

Studies on Iron Absorption

II Experiments with Iron deficient and Non deficient Rats

By

EINAR WOLFF SORENSEN

Our knowledge of what happens when iron is absorbed from the gastro intestinal tract is still limited

The *mucosal block* theory of Granick (6, 7) cannot explain the iron absorbtion in detail (3 4, 8, 13 18 19) The reaction Fe + apoferritin ⇄ Fe (fig 1) is reversible but it is not known what factors affect it How ferrous iron is transported into the mucosal cells is not clear The influence of dietary factors on iron absorption has been discussed by a number of authors (9 12 16 17 19 20) Many foods appear to aid utilization of dietary iron by reducing it or by maintaining it in the reduced state The literature seemed to lack detailed information about how protein carbohydrate and fat act on the absorption of ferrous iron and what effect additional administration of ethyl alcohol may have A study of the variations in serum iron in patients with iron deficiency anaemia indicated that iron absorption varied with different types of food (19) The effect of ascorbic acid on iron absorption seems to be complex Besides its reducing effect, ascorbic acid seems to have a specific influence on iron absorption (15b) It has not been possible to find any information concerning the effect of ethylalcohol on the iron absorption

The reliability of using a rise in serum iron as a measure of iron absorption is debatable The serum iron reflects different counter acting transport mechanisms and might therefore be misleading The purpose of the following experiments was to study by means of radioactive iron the influence of glucose fat ascorbic acid and alcohol on the absorption of ferrous iron in iron deficient and non deficient rats

Material and methods

1 Animals Female Wistar strain albino rats approximately 3 months old were used From 3 weeks after birth they had been separated into two groups A and B Group A had been

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Book review

The History of Surgical Anesthesia by Thomas E Keys, A B, M A With an introductory essay by Chauncey D Leake, a concluding chapter *The future of anaesthesia* by Noel A Gillespie and an appendix by John F Fulton 193 pp, 43 ill Dover Publications, Inc, New York 1963 Price $ 2 00

The head librarian of the Mayo Clinic, Thomas E Keys, a well known name in medical history circles, has brought out a revised and extended edition of his book which was first published in 1945 It is a meticulously thorough work which bears eminent witness to its author's great erudition Everything of value on the subject is presented in an attractive form, with reproductions of documents of particular value and of a number of pictures and portraits

In an appendix by John F Fulton the reader is introduced to *The Morton and Warren Tracts on Ether (Letheon)*

The view generally held hitherto that Priestley was the discoverer of oxygen and that Scheele made the discovery simultaneously and independently is repeated here A definite standpoint in Scheele's favour is not likely to come about until the first volume of Uno Boklund's work, *Carl Wilhelm Scheele — his life and work*, appears, probably this autumn

In a future edition the Swedish Xylocain, which was synthesized by Erdtman and Lofgren in 1943 and is used throughout the world, should have its given place, as should too its successor Citanest

One reads the book with great pleasure Packed as it is with information of different kinds, it can be used to advantage as a book of reference and should not be missing in any hospital library nor on the shelves of anyone who is interested in the history of medicine

Birger Strandell

Stockholm

TABLE II Weights and haemoglobin values for the iron-deficient and the non-deficient rats

Rat No	Non-defic ent rats Hb (g %) I	II	III	Weight (g)	Non-defic ent rats Hb (g %) I	II	III	Weight (g)
1	8 7	10	9 5	218	16	15 8	15 9	385
2	5 9	7	6 8	170	17.2	17	16 8	410
3	6 4	8	7	182	15 5	16	16 5	433
4	7 7	8 6	8 9	180	15 8	16	16	34?
5	5	5 5	6	195	17 3	17	17 1	430
6	7 2	8	7 3	214	16 5	16 7	16 5	416
7	5 2	6 2	6 4	180	14 7	15	15 1	355
8	5 2	5 6	5 0	174	16	16 1	16 3	450

I II and III indicate respectively that the estimations were performed before the experiments started when half finished and finished

beakers The meals were of different composition in each experiment

Exp I and IV 1 ml of the prepared iron mixture
1 g of a test meal

Exp II and V 1 ml of the iron mixture
1 g of a test meal
2 mg ascorbic acid

Exp III and VI 1 ml of the iron mixture
1 g of a test meal
0 5 ml 96 % ethyl alcohol

Experiments I II and III were on anaemic rats IV V and VI on non anaemic rats

Each experiment included 4 experimental periods of 8 days and each pair of rats received doses of iron combined with each of the different types of test meals according to a scheme in which the sequence of meals was arranged by a random selection procedure (table IV)

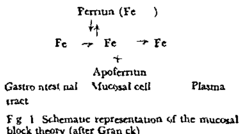


Fig 1 Schematic representation of the mucosal block theory (after Granick)

TABLE III Mean percentages of radioactivity left in the 8 rats in each test group 8 days after administration of radioactive iron

Experiment	Test meals F	C	P	Fasting
1 Iron-deficient rats given iron	70 1	72 3	51 7	60 3
2 Iron-deficient rats given iron and alcohol	81 3	72 2	0 2	74 2
3 Iron-deficient rats given iron and ascorbic acid	81 4	72	73 6	0 3
4 Non-deficient rats given iron	19.8	19 6	11 5	10 9
5 Non-deficient rats given iron and alcohol	10 4	13 4	9 1	7 8
6 Non-deficient rats given iron and ascorbic acid	9 8	14 9	7 2	6 6

TABLE I Rat diets The iron deficient diet contains the iron free salt mixture (designated diet 1) The iron supplemented diet contains the iron supplemented salt mixture (designated diet 2)

General composition of the diets	
Spray-dried skimmed milk	650
Sucrose	205
Vitamin mixture (see 1))	10
Salt mixture (see 2) or 3))	30
Choline dihydrogen citrate	5
Fat soluble vitamin mixture 4)	1
Fat	99
	1 000 g

1) Vitamin mixture	
Calcium pantothenate	1 2
Pyridoxine	0 5
Riboflavin	0 5
Aneurin	1 0
Nicotinic acid	1 0
Vitamin K	0 1
Folic acid	0 1
p-Aminobenzoic acid	1 0
Biotin	0 02
Inositol	20 0
Vitamin B_{12}	0 0015
Dextrose	974 577
	1 000 g

Salt mixtures

2) Iron free salt mixture	
Sodium chloride	200
Sodium iodate	0 022
Manganese sulphate	10 812
Copper sulphate	2 722
Dextrose	786 444
	1 000 g

3) Iron supplemented salt mixture

54 089 g of dextrose in mixture 2) has been replaced by 54 089 g ferrous ammonium sulphate

4) Fat soluble vitamin mixture	
Vitamin A palmitate	0 4 g
Calciferol	0 05 g
a Tocopherol	50 g

fed on an iron-deficient diet (diet 1 table I) and group B on the same diet supplemented with ferrous ammonium sulphate (diet 2 table I) The haemoglobin and the weight of the rats were recorded before the experiments Haemoglobin was also recorded during and after completing the experiments (table II)

2 *Test meals* Four types of test meals were prepared

1 Water = 0 (fasting ')
2 0 5 g dried skimmed milk powder = P (protein meal")
3 0 5 g glucose = C (carbohydrate meal)
4 1 g 35 % cream = F (fat meal)

All meals were mixed with water up to a volume of 2 ml

3 *Iron* Fe^{59} was obtained from the Radio-Chemical Centre Amersham England as ferric chloride of high activity (approx 5 $\mu C/\mu g$ Fe) Freshly prepared solutions of ferrous ammonium sulphate were used as carrier iron Each ml of the administered iron mixture contained 0 025 mg Fe and 0 5 μC Fe^{59}

4 *Counting* A scintillation scaling unit type 1009 D from Dynatron Radio Ltd Maidenhead England was provided with a phosphor well for whole body counting The rats were placed in cardboard cartons such that scintillation counting could be performed whenever required

The radioactivity left in a rat 8 days after oral administration of Fe^{59} was taken as a measure of the amount of absorbed iron This is valid since less than 1 % of an orally administered dose of iron can be found in the gut after that interval even in anaemic rats (1)

The rats both in the anaemic and in the non anaemic group were separated into pairs and the animals in each pair accompanied each other during all experiments performed

Except on the days when further doses of radioactive iron were given the rats remained on their primary diet On the experimental days after being starved from the previous day the rats were given meals prepared in small narrow porcelain

TABLE V The statistical analysis of the results of iron absorption The letters C O P and F refer to the amount of iron absorbed when these respective types of meals were administered

Experiment No	Significance		Experiment No	Significance	
	1% < P < 5%	P < 1%		1% < P < 5%	P < 1%
I	C–O	P–F P–C	IV	C–P	F–P F–O C–O
II	F–P	—	V	—	—
III	F–O	—	VI	C–O	C–P
I–II	F–F	P–P O–O	IV–V	—	F–F
I–III	F–F	P–P	IV–VI	—	F–F
II–III	—	—	V–VI	—	—

ed in experiment V In experiment VI C is found different from P An interesting point is the difference between F—F in experiments IV V and VI As for the comparison between experiments II and III no differences are found between the results in experiments V and VI

Between 2 and 3 weeks after these experiments were completed a further experiment was made Both the iron deficient and the non deficient rats were separated into two groups each consisting of 4 rats One group of the iron deficient and one group of the non deficient rats were given a glucose meal and the other two groups a protein meal All groups received ferrous ammonium sulphate and Fe^{59} according to the scheme mentioned earlier This time the rats were fed by means of a stomach tube during light ether anaesthesia The rats were subjected to whole body scintillation counting before and after feeding Between 1 and 1 1/2 hours later the rats were anaesthetised again The abdomen was opened and as much blood as possible was drained from the abdominal aorta The proximal part of the duodenum was taken out cut up and washed in normal saline The proximal 10 cm of the duodenum was put into tubes for radioactive counting Because of technical failure, the experiment with the non deficient rats had to be interrupted

Table VI shows the results of the radioactivity countings of the iron deficient rats There is no difference in the radioactivity of the blood between the two groups of rats (the high counts are due to the previously given doses of Fe^{59}) The radioactivity of the plasma from rats that received glucose is about double that which is found in the protein group The radioactivity of the duodenum from the glucose group is nearly double that found in the protein group Expressed as a percentage of the given dose the radioactivity of the

TABLE IV The applied randomly selected sequence of meals

	Rat No			
	1–2	3–4	5–6	7–8
1	O	P	C	F
2	P	F	O	C
3	C	O	F	P
4	F	C	P	O

O = fasting (no food)
P = protein rich meal
C = carbohydrate rich meal
F = fat rich meal

Results

Table III shows the mean percentages of radioactivity left in the eight rats in each test group 8 days after administration of the radioactive iron The difference in absorption of iron between the iron-deficient and the non deficient rats is pronounced The absorption of iron seems to be considerably higher in both groups when glucose or fat is administered compared with protein or no food at all These differences are greatly reduced by the addition of ascorbic acid or alcohol, and the effects of these two components are very much the same When given to the non-deficient rats both alcohol and ascorbic acid *increase* the iron absorption When given to non deficient rats, the absorption seems to be *decreased*

The results have been statistically analysed by means of ' analyses of variance for multiple classification ' and ' least significant difference ' (5) By the first of these tests the observed differences in iron absorption were related to variation in types of food, to the different rats and the different weeks For the *iron-deficient* rats, the observed differences in absorption in relation to the different types of food were found to be significant (**) with a level of probability (P) equal to 1 % For the *non deficient* rats the differences were found to be almost significant (*) with a level of probability between 1 and 5 % It should be stressed that these results are based upon 96 experiments in each of the two groups of rats (12 experiments on each rat)

The ' least significant difference ' test revealed between which of *the meals* the differences were most pronounced

The results have been tested against each other both *within* each experiment and also *between* the experiments (for the iron deficient and the non deficient rats respectively) In table IV the letters C O P F refer to the amount of iron absorption when these types of meal were administered

In the *iron deficient* rats the differences between P and F and P and C are highly significant This degree of significance is not found in experiments II and III A comparison between the results in experiments I and II show a high degree significance between P and P, and also between O and O When the results in experiment I and III are compared a highly significant difference is found between P and P, but between the results in experiment II and III no significant differences are found In the same manner, the results concerning *the non deficient* rats can easily be seen from the table In experiment IV F is different from P and O, and O is different from C but these differences are eliminat

Summary

In order to invest gate tl e influence of d fferent types of food on the absorpt on of ferrous iron a number of ron deficient and non defic ent rats were given ran domly selected meals consist ng of prote n carbol ydrate fat or water together w th Fe*

I on defic ent ats

A Compared w th prote n and water carbohydrate and fat increase the iron absorpt on cons derably There is no s gn ficant d fference n effect between water and prote n

B Wl en ascorb c ar d or ethyl alcohol s g en in add t on to prote n or vater the ron absorpt on is *ncreased* No s gn ficant ncrease is found w th tl e same add t on to carbohydrate or fat

C No d fference n effect s found be tween ascorl c ac d and ethyl alcohol

D In the prox mal 10 cm of the duode num and n the plasma app oximately 100 more rad oact ty is found when the rats ha e been fed on glucose than hen fed on protein

No def ent rats

A The amount of absorbed ron s con s derably less tl an in tl e ron def c ent g oup b t d t nctly h gl er when the ro s g en together v th glucose or fat tl a l en g en prote n or water

B A o l c ac d and ethyl alcohol both te d to decrease tl e ab orpt on irre spec e of tl e type of meal

C No d ffe ence n effect s found be twee a corb c ac d and ethyl alcohol

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TABLE VI The radioactivity of the anaemic rats after administration of glucose — or protein — and ferrous ammonium sulphate and Fe^{59} The radioactivity expressed as counts pr 2 minutes

Rat No	Meal	The radioactivity Total radio-active dose	In 4 ml blood	In $2^1/_2$ ml plasma	In 10 cm duodenum	The activity of the duodenum as percentage of the total dose
1	Glucose	53 879	182 306	5 930	15 424	28 6
2	Glucose	61 857	218 916	5 865	19 404	31 4
3	Glucose	57 220	196 585	5 078	15,240	26 6
4	Glucose	39 600	229,954	5 969	21,592	31 8
5	Protein	47 871	185 827	2 469	10 016	20 9
6	Protein	57 941	196 463	1 674	7 560	13 0
7	Protein	62,821	203 816	1 803	8 284	13 2
8	Protein	67 518	199 664	2 224	8 728	12 9

duodenum in the protein-group is approximately half of that found in the glucose-group

Discussion

Previous investigations (19) with iron-absorption tests on patients with iron-deficiency anaemia gave some indication that the amount of iron absorbed from the gut was dependent on the type of food given together with the iron The present experiments seem to support these earlier investigations

When iron is given to *iron-deficient* rats, carbohydrate and fat feeding gives a significantly higher iron absorption than protein feeding No difference is found between carbohydrate and fat in this respect The difference in effect between carbohydrate/fat and protein/no food is eliminated when ascorbic acid or ethyl alcohol is added No difference in effect was found between these two components The radioactivity of the blood, plasma and duodenum of the iron deficient rats, measured after administration of radioactive iron together with glucose or protein to different groups of rats, corroborates the former results The duodenal radioactivity is about 100 % higher in rats fed with glucose than in rats fed with protein, although higher radio activity is found in the plasma from the glucose rats' than in that from the protein rats' This indicates that more radioactive material has been transferred from the gut wall to the plasma in the former group When iron is given to *non deficient* rats, the amount of iron absorbed is far less than in iron deficient rats Moreover in these rats the amount of iron absorbed varies with the type of food present in the gut in the same manner as in the iron-deficient rats Addition of ascorbic acid or alcohol to the meals *decreases* the absorption especially in connection with fat meals No significant difference in effect is found between alcohol and ascorbic acid

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Orthostatic ECG Changes and the Adrenergic Beta-receptor Blocking Agent, Propranolol (Inderal)

By

Olof Nordenfelt

The ECG change occasionally seen in young persons with healthy hearts when they stand up are attributed by many authors (6 9, 12, 15) to augmentation of sympathetic tone associated with the effort of standing

These changes can be inhibited quite easily by administering 0 5 mg ergotamine tartrate intravenously (12) However as ergotamine tartrate has a complex action the results support the sympathetic theory but do not definitely establish its validity

In order to find a sympathetic inhibitor of greater specificity trials were undertaken with Hydergine (11) However this agent gave both a poor depression of the heart rate and a poor inhibition of the orthostatic ECG changes

This result which was difficult to understand in the light of the sympathetic theory appeared to be explained by the studies performed by Ahlquist in 1948 (1) This author separates the receptors of the sympathetic nervous system into alpha and beta receptors

The known sympathetic inhibitors, such as Hydergine, mainly blocked the alpha receptors and therefore had little effect on heart function or naturally on the ECG According to Black and Stephenson (5) Pronethalol (Nethalide Aderlin) is a specific beta receptor blocking agent Unfortunately the results of prolonged animal trials showed that the substance has carcinogenic properties and is therefore unsuitable for long-term therapy

A closely related substance recently developed, Propranolol (Inderal), is also a pure beta receptor blocking agent according to pharmacological investigations performed by Black et al in 1964 (4) It is about 10 times more effective and has so far shown no carcinogenic action

This substance has been used to study the cause of orthostatic changes in the ECG

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after intravenous injection of 5 mg of propranolol

Standing position					
Before			After i v injection		
ECG	Heart rate	B P	ECG (=recumb)	Heart rate	B P
Orth change II	110	110/75	Before	85	80/65
Orth change II	110	105/80	Before	90	95/?
Orth change I	100	105/80	Before	80	95/75
Orth change II	100	115/90	Before	75	105/90
Orth change I	100	100/80	Before	85	100/?
Orth change I	120	100/85	Before	95	95/85
Orth change II	95	120/90	Before	85	100/80
Orth change I	95	110/60	Before	70	95/60
Orth change II	125	—	Before	100	120/110
Orth change I	115	120/80	After	80	115/80
Orth change II	110	110/90	After	85	100/90
Orth change II	130	110/90	After	95	105/85
	110	110/82		83	100/82

Orth change II = Orthostatic ECG changes grade 2
after administration of propranolol

patient was required to stand for 5 minutes before lying down 12 leads were attached (I—III AVR AVL AVF V_1—V_6) Whilst the patient was standing he was given an injection of 5 mg propranolol (Inderal) and in some cases the ECG was recorded continuously for 5 minutes (leads I—III V) ECG recordings of all 12 leads were then made in both standing and recumbent positions

The blood pressure was taken repeatedly with the aid of an mercury manometer In standing position the arm was placed on a table at the height of the 3rd intercostal space

The heart rate was calculated from the ECG and is approximated to the nearest 5 beats

Results

Table I constitutes a summary of the results of the study

After i v injection of 5 mg propranolol an obvious reduction of the heart beat rate from 83 to 68 beats per minute was observed in all patients in the recumbent position The effect on the blood pressure was negligible The average reduction of the systolic pressure was from 124 to 115 mm Hg This reduction is difficult to evaluate since the patients were often nervous and agitated at the beginning of the examination The diastolic pressure was unaltered

In the 8 patients with normal recumbent ECG's recordings made in this position after the injection seldom showed any changes A slight increase in the height of the T-deflection in the lead representing the left ventricle was observed upon occasion

However in the 3 patients showing depressed T deflections before the injec

TABLE I Twelve patients with orthostatic ECG changes but no organic heart disease, before and

Case	Sex	Age	Diagnosis	Recumbent position					
				Before			After i v injection		
				ECG	Heart rate	B P	ECG	Heart rate	B P
1	♀	17	Cephalalgia	Normal	80	110/75	Normal	60	110/75
2	♀	42	Colitis ulcerosa	Normal	90	110/80	Normal	85	100/75
3	♀	21	Cephalalgia	Normal	60	125/80	Normal	55	105/75
4	♀	28	Vertigo	Normal	70	120/80	Normal	60	—
5	♀	19	Diabetes	Normal	85	120/90	Normal	70	115/90
6	♀	24	Struma atoxica	Normal	85	110/75	Normal	80	110/85
7	♂	21	Diabetes	Normal	65	145/90	Normal	50	115/80
8	♀	22	Observation	Normal	75	115/60	Normal	65	110/60
9	♂	22	Pneumothorax	Normal	85	145/100	Normal	60	135/100
10	♀	30	Neurosis	= Orth change I	100	130/90	Normal	75	120/85
11	♂	31	Neurosis	= Orth change I	95	135/85	Normal	70	125/90
12	♀	20	Neurosis	= Orth change I	110	120/80	Normal	80	120/85
Mean		25			83	124/82		68	115/82

Orth change I = Orthostatic ECG changes grade I
= recumb before or after = same appearance as ECG recorded in the recumbent position before or

Material and methods

Twelve patients (8 women and 4 men) with orthostatic ECG changes were examined Ten of these patients were hospitalized for various diseases (table I) two (Nos 8 and 11) were under ambulatory observation for heart conditions The ages of the patients ranged from 17 to 42 years the mean age being 25 years

No heart disease could be established in 9 patients (Nos 1-9) and the ECG registered in the recumbent position was perfectly normal The remaining 3 patients (Nos 10—12) sought medical advice for palpitation and were nervous and uneasy Physical examination and X rays did not reveal any heart disease However recumbent ECG s were not entirely normal The T deflections of the II—III and V_4—V_7 leads were very low and some were diphasic A further characteristic was the spontaneous change in the appearance of the ECG on different occasions When the patients were especially nervous sinus tachycardia was often recorded and there were greater variations in the T deflections

In order to simplify the description of the orthostatic ECG changes in table I they have been divided into two degrees

Grade 1 1—3 mm depression of the T deflection on one or more of the leads II—III AVF V_4—V The T wave was not negative or diphasic

Grade 2 The same changes as for grade I but also negative or diphasic T deflection generally in III II or V

Sinus tachycardia 95—150 beats/min (mean 110/min) was recorded in all the patients and there was an increase in the height of the P-deflection

No marked S—T depression was observed in the patients

The examination was performed in the following manner

The instrument used was a direct recording 4-channel electrocardiograph (Elema The

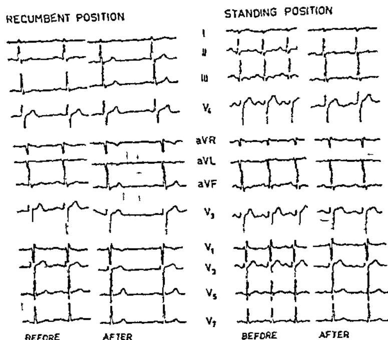


Fig 3 Case 9 Intravenous injection of 5 mg propranolol (Cf table I)

tion there was a considerable change in the ECG recorded after the injection All the T waves attained a normal height and the ECG had an entirely normal appearance (fig 1)

A marked reduction of the heart rate was observed in the standing position even after the administration of propranolol on the average from 110 to 85 beats per min In comparison with the values obtained in the recumbent position the systolic blood pressure fell from 124 to 110 mm Hg and fell even further after the injection to an average of 100 mm Hg The diastolic pressure however remained unchanged although this was sometimes rather difficult to determine as the sounds were weak and on two occasions the recordings were invalid

After injection of propranolol, the orthostatic changes in the ECG disappeared for the most part (figs 2 and 3) The ECG became normal and its appearance was almost identical with that recorded in the recumbent position In the case of the three patients (Nos 10, 11 and 12) with abnormal recumbent ECG s before injection, the ECG s had almost the same appearance as those recorded in the recumbent position after the injection

Curves representing the heart rate, the height of the T-deflection in the leads II III and V_4, and the height of P_{II} have been drawn for the three patients (Nos 4 10 and 12) for which ECG s were recorded continuously in the standing position during the first 5 minutes following the injection (fig 4)

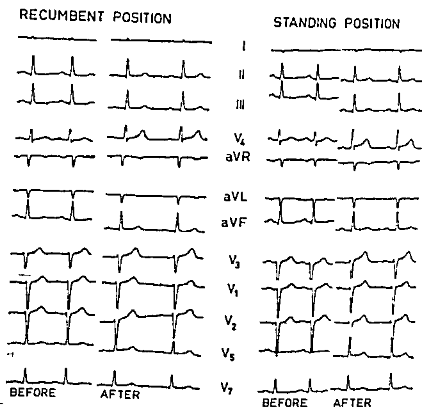


Fig 1 Case 11 Intravenous injection of 5 mg propranolol (Cf table I)

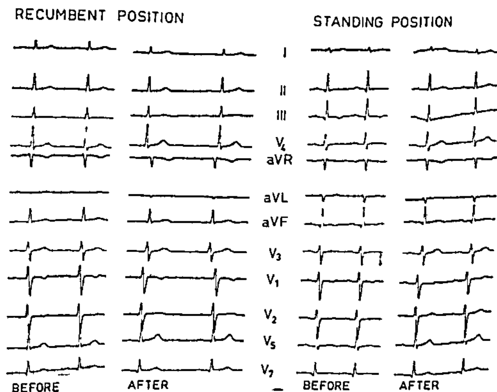


Fig 2 Case 1 Intravenous injection of 5 mg propranolol (Cf table I)

ical (13) that although it is possible to slow the heart rate considerably in the standing position by massage of the carot id sinus the orthostatic changes in the ECG remain unchanged Further, the fact that the reduction in the heart rate after administration of propranolol oc curs earlier than the elevation of the T-deflections (fig 4) may support the assumption that these phenomena are not directly dependent upon each other On the other hand, the P-deflections appear to follow the beat rate rather closely

Contrary to the results of ergotamine injections there was no rise in blood pressure following the intravenous injec tion of propranolol In the recumbent position the systolic pressure fell slightly from 124 to 115 mm Hg on the average, and in the standing position from 110 to 100 mm Hg Thus the normalization of the ECG changes cannot be attributed to an improvement in the blood pressure

The assumption that coronary insuffi ciency is the cause of the negative T de flections (17) is hardly acceptable for this material which consists mainly of young individuals (mean age 25 years) in whom organic heart changes could not be confirmed

Apart from the ECG changes espe cially QRS arising from the altered posi tion of the heart in the standing position, the present study in which the adrenergic beta receptors of the heart are blocked appears to offer evidence that *the orthostat ic changes in the ECG are a direct result of the change in the working method of the heart which in turn is caused by the increased sympathetic tone*

In patients 10 11 and 12 it was shown that even ECG changes arising in the recumbent position are affected by beta receptor blocking agents These particular patients were nervous people whose ECG s, recorded at rest showed spontaneous changes indicative of temporary and benign or functional ECG changes The absence of these changes after the administration of beta receptor blocking agents indicates that they are caused by the increased sympathetic tone and might therefore be designated orthostatic changes in the ECG registered in recumbent position'

These patients expressed their relief at the slower heart rate and felt that the unpleasant sensation in the chest had disappeared They felt calmer and safer It would therefore appear to be of great value if a material comprising this type of patient could be gathered together and trials performed to find out whether an adrenergic beta receptor blocking agent taken orally when necessary or con tinously would provide a more enduring relief from their distress which frequently assumes large proportions and causes invalidity

The fact that adrenergic beta receptor blocking agents normalize sympathicotonic changes in recumbent ECG s leads us to hope that such an agent would be highly useful in cases which the diagnosis is difficult to confirm

Unfortunately, there are pitfalls which must be taken into account

Since sympathectomy has a favourable effect on patients suffering from angina pectoris adrenergic beta receptor blocking agents should have a similar effect. Schroder and Werko (14) have demon strated that the energy requirement of the heart after pronethalol (Aderlin)

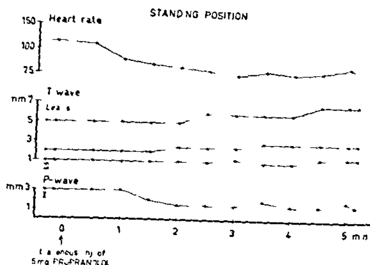


Fig 4 Case 10 The curves are plotted for the heart rate the T wave and the P wave, taken from continuous ECG recorded in the standing position immediately prior to and concurrently with the intravenous injection of 5 mg propranolol (Cf table I)

It can be seen that the heart rate begins to fall after 1/2 min, and the maximum fall is reached 2—2 1/2 min after the intravenous injection The fall in the height of the P-deflection follows closely the drop in the heart rate The rise in the T-deflections, however, is slower reaching the maximum after 4—5 minutes

Attempts were made with two patients to determine the time when the effect of the injection wore off After 20 min, patient 2 was given a further 5 mg of propranolol which resulted in a prolongation of the P Q-time to 0 32 sec This prolongation of the heart activity reverted to normal after 2 1 2 hours, the effect of the injection having probably worn off by that time In patient 10, ECG's were recorded in standing and recumbent positions every hour after the injection The heart rate after somewhat more than two hours, recorded standing was 100 per min, and orthostatic changes in the ECG began to become apparent, presumably a sign of the fading effect

The patients experienced remarkably little subjective distress after the intravenous injection of propranolol Despite the prolongation of P-R interval in one patient and the fall in the systolic blood pressure to 95 mm Hg while standing in patients 2, 3, 6, and 8 and to 80 mm Hg in patient 1, there were nearly no complaints of dizziness or other symptoms of distress The ECG's for patients 10, 11 and 12 were not normal when recorded in the recumbent position before the injection but these patients stated unanimously that they became calmer and experienced far less cardiac disturbance after the injection

Discussion

The investigation has shown that when 12 patients exhibiting orthostatic ECG changes were given intravenous injections of 5 mg propranolol these changes became blocked and the ECG's had a normal appearance

Since this normalization occurred concurrently with a reduction in the heart rate, it is necessary to reconsider the assumption made by Sjostrand (15) that the changes in the beat rate *per se* caused the changes in the ECG It was shown in an earlier article in this period

changes are due to the increased sympathetic tone inherent in the standing position

Since 3 of the patients participating in this study exhibited recumbent ECG changes resembling orthostatic changes, the possibility of using beta receptor blocking agents for diagnostic purposes was discussed As a result of the injection, the patients stated that they felt calmer and that their subjective heart trouble disappeared These observations suggest the possibility of treating this type of patients with this substance

Now that we know how the sympathetic affects the ECG it might be proposed that all test subjects receive pre treat ment with beta receptor blocking agents when determining the normal values for the height of the P and T-deflections

It is also proposed that healthy test subjects with orthostatic ECG changes are suitable objects of study when the effect of adrenergic beta receptor block ing agents is being investigated

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administration is less, both for normal test subjects and for hypertensive patients. It has been shown using both pronethalol (2, 3, 10, 8) and propranolol (10,17) that these substances diminish anginal distress and even inhibit the S-T depression of the working ECG.

The possibility exists, therefore, that the ECG changes caused by coronary insufficiency in patients with coronary sclerosis become reduced or disappear after the administration of beta receptor blocking agents.

Studies performed by Carlsten in 1963 (7) have revealed another pitfall. This author studied patients with changes of myocarditis in the ECG. When the myocarditis began to heal and the recumbent ECG became normal, Carlsten was able to show that the changes due to myocarditis reappeared on ECG's taken in the standing position. In such cases, it is possible that the inhibition of the sympathetic tone by beta receptor blocking agents may lead to the faulty diagnosis of functional ECG changes.

However, if these pitfalls are kept in mind, there appears to be a number of instances when an adrenergic beta receptor blocking agent would be a considerable aid to the diagnosis of doubtful cases.

Since the sympathic action on the heart sometimes deviates considerably even in the recumbent position, as in patients 10, 11 and 12, the question may be raised whether this point should not be given greater consideration when calculating and determining the normal variation range for the height of the P- and T-deflections. It might be advisable when recording ECG's to administer adrenergic beta-receptor blocking agents to the entire normal material and later, when taking diagnostic ECG's, always to give the patients beta receptor blocking agents if the P- or T wave heights exceed the new normal values.

Finally, this investigation has demonstrated that healthy persons with orthostatic ECG changes are highly suitable subjects on which to test adrenergic beta receptor blocking agents. Continuous ECG recordings in the standing position after injection provide information on how and when the substance takes effect. By repeatedly registering ECG's in the standing position, it is possible to determine the time when the effect ceases since the orthostatic changes will then reappear in the ECG.

With regard to propranolol, the onset of effect occurs after 1/2—1 min and reaches its maximum after 4—5 min. The effect has faded entirely after approximately 2 hours.

Summary

The main purpose of this investigation has been to study orthostatic changes in the ECG by means of adrenergic beta receptor blocking agents.

The test material consisted of 12 patients exhibiting such changes but in whom heart disease could not be confirmed. After intravenous injection of 5 mg propranolol these changes disappeared to such a degree that the ECG presented a completely normal appearance.

Apart from the effect which a change in the position of the heart exerts on the ECG, it can be concluded *that any other*

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Local Skin Necrosis after Intravenous Infusion of Norepinephrine, and the Concept of Endotoxinaemia

A Clinical Study on 10 Cases

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Intravenous infusion of norepinephrine has since 1949 been used in the treatment of shock (15) Local skin necrosis at the site of the infusion has been reported in about 150 cases (5 7, 8, 9, 19 26, 27, 29 35 37 38, 45 and others)

The proposed causes of the necrosis were

a) Extravascular infusion of norepinephrine or paravenous infiltration through an injured vein (9, 10)
b) Passage of norepinephrine through an intact or ischaemic vessel wall 9 23 38)
c) Reflux of norepinephrine solution to smaller vessels with local concentration in arterioles and venules (4 7)
d) Local ischaemia due to for instance arteriosclerosis or vascular spasm before the start of norepinephrine infusion (25 41)
e) Thrombophlebitis and stasis after prolonged infusion (27, 38)

Since 1956 altogether 10 patients with skin necrosis after norepinephrine infusion have been admitted to this clinic These cases are here discussed with regard to endotoxinaemia as a possible causal factor in the necrosis

1) Thomas et al (39 40) have shown that intravenous injection of endotoxin in rabbits promotes the development of skin necrosis in association with subsequent intracutaneous injection of small amounts of epinephrine or norepinephrine
2) We reported in 1962 that intracutaneous injection of 0 1 mg of epinephrine produced a small patch of well defined local bluish purple discoloration of the skin in about 30 % of this clinic s patients with acute renal failure — a disease which in some cases may be due to endotoxinaemia (42) The primary disease in these patients was cholecystitis, peritonitis septicaemia, colitis etc Besides the symptoms known from endotoxin investigations in rabbits e g fever, leucocytosis, thrombocytopenia many of the patients with a positive epinephrine test also showed somno-

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Case 1 A 32 year-old woman tried to induce an abortion in the third month of pregnancy by introducing a knitting needle into the uterus (day 1) A chill and a temperature rise to 41 2° C occurred on day 3 Diarrhoea intercurred on day 4 Her pulse rate was 160 beats per minute, and B P 90/50 mm Hg Instrumental exaeresis and curettage were performed Urine secretion stopped A B P fall occurred 6 hours after the operation she became mentally confused and had cold perspiration with peripheral coldness and cyanosis In spite of prompt blood transfusion and intravenous hydrocortisone treatment her condition deteriorated with disappearance of peripheral arterial pulses B P rose to 80—100 mm Hg systolic in response to intravenous infusion of norepinephrine Culture of secretion from the uterus yielded growth of E coli

When the patient was transferred to this clinic on day 5 she had received althogether 1 800 ml of blood and 2 500 ml of glucose fructose solution with 14 mg of norepinephrine 1 400 mg of hydrocortisone as well as desoxycorticosterone and A C T H She had extensive petechiae on her trunk and face merging at the nose which was of a dark bluish purple colour Petechiae were also seen on the palete At the site of the norepinephrine infusion there were large patches of necrosis of the skin The patient was treated with the artificial kidney 14 times and lived until day 42 Norepinephrine was infused during the first 2 days in this clinic but local necrosis did not develop

Autopsy showed among other findings renal cortical necrosis and necrotic areas in the liver spleen adrenals and brain

Case 2 A 46-year old woman had had recurrent episodes of biliary colic and cholecystitis and cholangitis Cholecystectomy was made and a common-duct stone was exirpated Post-operatively the patient was jaundiced because of another concretion in the lower part of the common duct. A second choledocholithotomy (day 1) was troublesome the stone was extracted after 5 hours During the operation the patient s B P fell below 100 mm Hg systolic She was given a transfusion of blood plus norepinephrine About 1/2 l of blood passed outside the vessel in the right leg which became markedly swollen before the damage was discovered On day 2, she still had a tendency to shock. Anuria developed Her temperature rose to around 40° C

On admission to this clinic on day 5 the patient was somnolent and had superficial respiration Her skin was highly icteric. The abdomen was distended by gas and tender as in peritonitis On the right leg there was a bluish red swollen infiltration extending from the ankle to the groin Within this area on the lower leg were seen incipient necrosis and some fluid-containing blisters about 3 cm in diameter The patient was haemodialyzed 5 times During the first treatment she exhibited intense constriction of both the artery and the vein She developed peripheral cyanosis haematuria and melaena and died in shock on day 10

Autopsy showed, among other findings bile peritonitis tubular necrosis a septic spleen and "toxic liver injury

The bile ducts as well as the choledochus were distended The choledochus showed a small penetrating lesion

Case 3 In a 52 year-old woman a hysterosalpingo-oophorectomy was performed because of suspected carcinoma of the uterus Appendectomy was made at the same time Microscopical examination showed no malignancy of the uterus or adnexa but an adenocarcinoma of the appendix Radical operation in the ileocaecal region was performed 9 weeks later on day 1 when the patient was in a good general condition Microscopial examination of resection specimens showed no evidence of metastases. After this operation the patient passed large amounts of urine Diabetes mellitus was diagnosed on day 4 Insulin was given On day 5 her temperature rose to 40 C. On day 6 shock occurred for which she was given intravenous infusion of dextran plus norepinephrine. She passed greenish mucous stools. Oliguria and uraemia intercurred

TABLE I Diagnosis at the time of the norepinephrine infusion that caused necrosis

Case no	
1	Induced abortion sepsis, shock
2	Recurrent cholecystitis cholangitis, jaundice cholecystectomy-choledocholithomy laparotomy repeated shock
3	Adenocarcinoma in the appendix, ileocaecal resection suspected sepsis shock
4	Acute abdominal disease (laparotomy) peritonitis, shock
5	Cholecystolithiasis, cholecystectomy choledocholithomy acute pancreatitis shock
6	Perforated peptic ulcer (laparotomy (raphi)), peritonitis enteritis suspected sepsis, shock
7	Chronic alcoholism, delirium tremens, shock
8	Chronic prostatitis with concretions, stricture of urethra chronic pyelonephritis acute retention of urine (catheterization of bladder) periurethral abscess (vesicotomy) suspected sepsis shock
9	Cholelithiasis suspected gastric tumour (gastrotomy cholecystectomy) subphrenic abscess shock
10	Mild chronic polyarthritis acute severe enterocolitis shock

lence or coma peripheral cyanosis, vasoconstriction cold perspiration, pale and slightly yellow skin tachycardia and tachypnoea or respiratory failure Subsequent to this work on the cutaneous reaction to epinephrine, it was also shown (14) that norepinephrine injected intradermally in man may in some cases cause the same type of reaction as did the epinephrine injection

3) Greisman et al (17, 18), using a technique similar to ours, found that this reaction to catecholamines was present in cases with human typhoid fever

From this clinic Alwall and Kjellstrand (2) analysed 639 cases of acute renal failure" treated over the period 1946—1961

Patients with shock in association with hyperpyrexia at the time of the onset of acute renal failure made up 18 % of the material Shock in association with hyperpyrexia occurred for example in about 25 % in patients with biliary tract diseases (non-operated and operated) In the cases with a urological basic disease these symptoms were noted at the onset of acute renal failure in 50 % of the deaths and in 6 % of the survivors Hyperpyrexia, that is chills and temperature around 40° C, with or without shock occurred in 31 % of the total number of cases Shock resulting from bleeding at the time of the onset of acute renal failure occurred in 13 2 %

Case reports

The patients were prior to admission to our clinic selected as cases with acute renal failure complicating a primary disease The diagnosis at the time of the norepinephrine infusion that caused necrosis will be seen in table I

Four of the patients (cases 1 6 7 and 8) have been reported in detail earlier (1 p 152 143 177 and 153) with reference to the renal complication This description of these cases will concern the course of the disease especially at the time of the development of the norepinephrine necrosis. One patient (case 1) has also been described as representative of the generalized Shwartzman reaction in man (28)

The patient was transferred to this clinic on day 4 because of increasing N P N By then her skin was cold clammy and icteric A discoloured area, almost the size of the flat of a hand, was seen medially on the right leg after previous norepinephrine infusion Centrally in this area the skin was necrotic Bronchopneumonia developed and tracheotomy was performed

She became unconscious in the night before day 5 Respirator treatment was started In the afternoon of day 5 she died in irreversible shock

Autopsy showed among other findings duodenal ulcer acute pancreatitis and toxic changes in the liver, spleen, and kidneys

Case 6 A 26-year-old man fell ill with acute severe abdominal pain (day 1) At laparotomy performed immediately in another hospital several litres of fluid were found in the abdominal cavity A perforation of the stomach was sutured The duodenum was markedly changed and the surrounding tissue firmly fixed against the pancreas Closely distal to the pylorus there was another perforation The perforation was closed and covered with a flap from the lesser omentum The wound ruptured and was resutured on day 3 On day 8 the patient had a temperature rise copious diarrhoea and shock His B P returned to normal in response to intravenous norepinephrine Anuria developed

Copious diarrhoea persisted on admission to this clinic (day 10) N P N rose towards 170 mg 100 ml and dialysis was performed on day 13 Thereafter the urine volumes increased A necrotic wound after norepinephrine infusion was not ready for grafting until day 56 when the patient was discharged with good renal function

Case 7 A 34 year-old man was admitted to hospital day 1) with severe delirium tremens. On day 5 he suddenly had repeated attacks of generalized convulsions with cyanosis. He passed into coma and was severely shocked with at times unpalpable peripheral arterial pulses The shock was brought under control within 2—4 hours by intravenous infusion of fluid, hydrocortisone and norepinephrine in increasing concentration A few hours later, severe shock recurred and was gradually controlled as before. In the evening he had generalized convulsions and was hyperpyretic Oliguria and uraemia developed

The patient was transferred to this clinic on day 9 in a very poor general condition, highly cyanosed and with short sudden breath He was deeply comatose Tracheotomy was performed and respirator treatment started Haemodialysis combined with ultrafiltration was started in the night before day 10 In the course of repeated haemodialysis he improved slowly On day 35 he seemed mentally fairly clear and urine secretion increased He was discharged after 3 months stay in this clinic

Case 8 A 48-year-old man had repeated episodes of complete retention of urine due to stricture of the urethra and prostatic calculi The stricture was divided and a catheter a demeure was used for a week After removal of the catheter (day 1) he was able to pass water without difficulty On the morning of day 3 his temperature rose and shock occurred systolic B P was 65—80 mm Hg Dextran solution with norepinephrine was infused intravenously Urinary secretion ceased Cystotomy was made on day 4 with formation of a suprapubic fistula The bladder contained only 150 ml of urine Uraemia and jaundice developed The patient had a meteorically distended abdomen with paralytic ileus Discharge of 1 1/2 l of dark brown fluid through a duodenal tube indicated haemorrhage into the digestive tract.

On admission to this clinic on day 8 the patient was somnolent and mentally confused Serum bilirubin was 17 mg/100 ml N P N about 250 mg/100 ml The skin on his left forearm and dorsum of the hand was after norepinephrine infusion of a bluish purple colour over an area of about 4 × 12 cm, with two thrombotic veins and central necrosis Urine culture yielded profuse growth of B proteus and coliform rods. Repeated haemo-

On admission to this clinic on day 6, the patient was apathetic She had forced rapid respiration and peripheral oedema An area of skin necrosis, the size of the palm of a hand, was present on the tibial side of the right lower leg, at the site of the norepinephrine infusion The 1st to 3rd digits of the right foot were bluish cyanotic and cold, despite fairly good peripheral arterial pulses The pharyngeal mucosa was coated with slightly greenish purulent secretion The abdomen was slightly raised and gaseously distended but without rigidity or tenderness Chest X ray showed evidence of fluid retention and/or stagnation of secretion, and suspected pneumonia A tracheotomy was performed and respirator treatment started The patient's condition improved noticeably in response to these measures Staphylococcal sepsis was suspected, but blood cultures were negative Intensive chemotherapy was started The bluish cyanotic discoloration of the right foot gradually spread to all the digits of the foot and the distal quarter of the metatarsus became gangrenous Amputation was con sidered, but on the morning of day 9 severe shock occurred and the patient died within a few hours

Autopsy showed, amoung other findings, toxic ' injury of the liver, a septic spleen and renal tubular necrosis Endocardial bleedings were also noted

Case 4 A 59 year old woman fell ill with diffuse abdominal pain vomiting, and diarrhoea (day 1) She was afebrile and had a B P of 220/110 mm Hg when admitted to hospital X ray examination showed sus pected ileus, and explorative laparotomy was performed on day 3 Her abdomen was raised but soft An adhesion of the great omentum to the sigmoid colon was divided There were some oedematous proximal small intestine loops but no bands The colon gall bladder, and stomach were normal on palpa tion Post operatively, in the night before day 5, her B P fell to 70 mm Hg systolic Dextran solution plus norepinephrine infused intra venously maintained the B P around 110 mm Hg systolic Anuria developed In the afternoon of day 5 she had another B P fall and became mentally confused Besides other parenteral fluid therapy, dextran with norepinephrine and blood transfusions were given again

The patient was transferred to this clinic on day 7 She had a fixed staring gaze, moaned now and then, and did not answer when spoken to Marked peripheral cyanosis was noted Her skin was clammy Her left leg was slightly oedematous up to the knee, after previous norepinephrine infusion On the middle part of the leg there were a few watery blisters, about 2 × 3 cm, without surrounding local reaction The abdomen was greatly distended and no bowel sounds were heard on auscultation Lung ventilation was poor

Tracheotomy was made and respirator treatment started Haemodialysis was per formed She still needed intravenous norepi nephrine infusion Her skin was warm over the trunk and head but intensely cyanotic and cold over the arms and legs A few watery blisters resembling burns also appeared on the right lower leg and dark necrotic changes of the skin were seen in a 49 year old appendectomy scar The patient died in irreversible shock on day 9

Autopsy showed among other findings diffuse purulent peritonitis, distinct toxic ' injury of internal organs tubular necrosis and infarction of half the right kidney

Case 5 A 70 year old woman with arterial hypertension was cholecystectomized after a period of frequent attacks of biliary colic with transient jaundice The gall bladder which contained several stones perforated during the operation (day 1) Cholangiography showed a defect in the contrast medium at the papilla of Vater but no concretions were found at the choledo- chotomy Post operatively in the night be fore day 3 the patient developed pallor of the skin and cold perspiration Her B P fell towards 100 mm Hg systolic Oliguria arose Intravenous infusion of dextran plus norepinephrine raised the systolic B P to 150—160 mm Hg Haematemesis intercurred

Results

In 3 of the patients the shocks were complications of acute diseases in previously essentially healthy persons (cases 1, 3 and 4) In 4 (cases 2, 5 8, and 9) the shocks were complications of chronic diseases of the biliary or urinary tracts

The disease was in 3 cases of infectious type, chronic (cases 2 and 8), or acute (case 1) with verified or suspected septicaemia (cases 1 and 8) Severe infectious complication developed in another 4 cases (nos 3, 4 6, and 9) with septicaemia in 1 (case 9), as judged clinically and verified at autopsy

In none of our cases was the shock caused by blood loss, acute exogenous poisoning heart failure, or pulmonary insufficiency In 8 cases it was of a type most closely resembling septic or toxic shock (2, 12 44) Shock of obscure cause occurred during operation in 1 case (no 2) and during delirious convulsions in 1 case In 3 of the cases (nos 1 7 and 10) the shock was so severe that the blood pressure could not be measured peripherally Some laboratory and other data are set out in table II One patient (case 3 was admitted to this clinic on the same day as the shock occurred the rest were admitted within 1 day (case 1), 2 days (cases 4 5 9 and 10) 4 days cases 2 and 7) and 7 days (case 8) of the shock

Symptoms

Fever Six patients were febrile before the shock 3 (cases 1 3 and 9) had temperatures between 40 0 C and 41 2 C on the day before the shock All the 10 patients had rectal temperatures of at least 38° C during or soon after the shock, 5 had over 40° C One patient (case 7) became hyperpyretic a few hours after the shock

Leucocytosis Nine of the 10 patients had 12,000 or more white cells per mm³ in counts made within 3 days of the shock (table II)

Thrombocytopenia Three out of 8 patients had 70,000 platelets or less per mm³ as counted within 3 days of the shock In 2, the thrombocytes were not counted until 5—6 days after the shock, the numbers were then below normal One patient (case 1) had 9,000 thrombocytes per mm³ on the day after the shock

Mental disorders Restlessness and disorientation as well as somnolence and coma occurred Restlessness and/or mental confusion were noted in 5 patients (cases 1 4 7 8 and 10) immediately after the shock One patient (case 3) was somnolent on admission to this clinic on the day of the shock One (case 7) passed into deep coma immediately after the shock and 2 (cases 4 and 5) did so 2—3 days later in the course of the disease Two patients (cases 2 and 8) were somnolent on admission to this clinic 4—5 days after the shock In 2 cases (nos 6 and 9) cerebral disorders do not seem to have been present

Intestinal disorders such as diarrhoea or intestinal paralysis were in addition to the primary disease present at the time of the shock in 2 cases (nos 1 and 8) In all diarrhoea coincided with the shock in 4 cases (nos 1 3 6, and 10) and intestinal paralysis in 1 (no 8) Suspected ileus developed in 2 cases (nos 4 and 10)

dialyses were performed Tracheotomy was made on day 9 His general condition improved gradually N P N was normal on day 44 Urethrocystography later in the course showed that dorsally to the stricture there was an abscess cavity

On day 29, he had a grand mal attack. He died suddenly on day 31

Autopsy showed numerous abscesses localized to the pelvis, the subphrenic region, and the cerebrum, as well as tubular necrosis, toxic hepatosis, and a septic spleen

Case 9 A 59 year-old man had since the age of 37 years had epidodes of abdominal distention and abdominal pain At the age of 59, he was examined with X ray which showed gall bladder stones and a polypous gastric growth He was admitted to hospital for operation His general condition was good, B P was 200/100 mm Hg Gastrotomy and cholecystectomy were performed (day 1) No polyp was found in the stomach, there was only a large mucosal fold Microscopical examination of the gall bladder showed evidence of mild chronic cholecystitis The immediate post operative course was normal On day 4, his B P fell to 95 mm Hg systolic and his temperature rose to 40 5° C, his pulse rate was 125 per minute He was given norepinephrine, Aramine®, hydrocortisone and antibiotics Oliguria and uraemia developed

On admission to this clinic (day 6), the patient was in fairly good general condition His feet were cold and he had slight peripheral cyanosis The mucous membranes were pale and coated with small crusts Abdomen was slightly meteoric A vein at the ankle of the right leg had been exposed for infusion, on the medial aspect, approximately mid leg, there was a round patch of bluish black discoloration The leg was slightly doughy and oedematous Gradually, a patch of necrosis measuring 4 × 4 cm developed over the discoloured area Dialysis was performed on days 7, 9, 11, 14, 16 20 and 29 Tracheotomy was performed on day 8 because of hypoxaemia Respirator treatment was started on day 10 Staphylococcal septicaemia, reasonably arising from peritonitis, was established on day 15 Large amounts of chemotherapeutic drugs were given and improvement was noted Daily urinary output increased gradually to 2 1/2 litres As from day 21, he did not need the respirator

Case 10 A 64-year-old man with mild joint pain had been treated with small doses of cortisone In the night before day 1 he suddenly became very ill with nausea and vomiting Diarrhoea with viscid and blood stained discharges developed

On admission to hospital on day 1, his hands and feet were bluish cyanotic He was cold and did not perspire The superficial veins had collapsed, arterial pulses could not be felt peripherally He had motor restlessness and slight clouding of consciousness Norepinephrine infusion raised his B P to above 100 mm Hg systolic In the afternoon of day 2, it was suspected that norepinephrine had been infused extravascularly B P was thereafter maintained with Aramine® Oliguria was present and uraemia arose Abdominal distention developed gradually

On admission to this clinic on day 3 he still had marked peripheral cyanosis On the medial aspect of his left lower leg there were red discoloured patches extending from 1 cm above the ankle towards the knee X ray examination showed dilatation of the small intestine mechanical ileus was suspected Explorative laparotomy showed a dilated proximal part of the small intestine The distal part was of normal width No mechanical obstruction was found Blood-culture on day 3 yielded no growth but Pseudomonas grew in blood cultures on days 11 and 13 Daily urinary output increased however, and no haemodialysis was made N P N became normal by degrees although the diarrhoea persisted Repeated cultures showed no salmonella or shigella infections Examination of the rectum showed a swollen slightly bleeding mucosa with patches of fibrin deposits and a few small erosions The patient recovered gradually but had signs of myocarditis later in the course

Thrombocytes × 10 (day)	White-cell count × 10³ (day)	Necrosis		Outcome (day)
		Localisation	Size	
12 (ə) 10 (6)	7 0 (4) 39 4 (6)	Lower leg	Large	Dead (42)
,0 (4) 72 (8)	51 0 (2) 31 9 (3)	Lower and upper leg	Large[1]	Dead (10)
52 (6 ɔ6 (7	12 1 (6) 25 5 (7)	Lower leg	Medium[1] (7 × 12 cm)	Dead (9)
91 (7 1'8 8)	12 6 (7) 26 2 (8)	Abdominal scar	Small[1]	Dead (8)
120 4) 150 5)	22 2 (4)	Lower leg	Medium (7 × 12 cm)	Dead (ɔ)
1'6 10	26 8 (9) 28 9 (10	Lower leg	Medium[1] (7 × 12 cm)	Alive
((10 181 12	8 5 (10 8 9 (12	Upper leg	Medium	Alive
4? 9 3(10	14 0 (4) 29 8 (6)	Upper leg	Medium (4 × 12 cm)	Alive
114 (6	13 3 (6	Lower leg	Small (4 × 4 cm)	Dead (31)
1(0 3 (4	16 4 (3 12 1 (4)	Lower leg	Large	Alive

[1] Gangrene of the foot developed

TABLE II Some laboratory and other data

Case no	Sex	Age (years)	Day of shock	Day of arrival	Some clinical data at arrival to the renal clinic
1	♀	32	4	5	Vasoconstriction Respiratory insufficiency BP 70/30, P 120, T 41.2° C
2	♀	46	1	4	Vasodilatation Superficial respiration Somnolence BP 125/85, P 124, T 37.5° C
3	♀	52	6	6	Vasoconstriction Respiratory insufficiency Somnolence BP 130/80, P 140, T 38.0° C
4	♀	59	5	7	Vasoconstriction Respiratory insufficiency Coma BP 100/60, P 140, T 39.3° C
5	♀	70	2	4	Vasoconstriction Respiratory insufficiency Confusion BP 100/60, P 104, T 37.9° C
6	♂	23	8	10	BP 90/60, P 100, T 37.7° C
7	♂	34	5	9	Vasoconstriction Respiratory insufficiency Delirium, coma *BP 130/85, P 55, T 36.8° C*
8	♂	18	3	8	BP 150/90, P 90, T 37.7° C
9	♂	59	4	6	Vasoconstriction BP 150/90, P 100, T 37.7° C
10	♂	64	1	3	Vasoconstriction Confusion BP 115/80, P 88, T 36.3° C

[1] Vesicles up to 2 × 3 cm surrounded the necrosis

[2] Vesicles developed on both legs close to the site of norepinephrine infusion

Thrombocytes × 10³ (day)	White cell count × 10³ (day)	Necrosis		Outcome (day)
		Localisation	Size	
12 (5) 10 (6)	7 0 (4) 39 4 (6)	Lower leg	Large	Dead (42)
70 (4) ɪ2 (8)	ɔ1 0 (2) 31 9 (3)	Lower and upper leg	Large[1]	Dead (10)
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91 7 1 8 8	12 6 (7 26 2 (8)	Abdominal scar	Small[4]	Dead (8)
320 4 150 5)	22 2 (4	Lower leg	Medium (7 × 12 cm)	Dead (5)
1 6 10	26 8 (9 ?8 9 (10	Lower leg	Medium[4] (7 × 12 cm)	Alive
ɪ (10 181 12	8 5 (10 8 9 12)	Upper leg	Medium	Alive
42 9 36 10	14 0 (4) 23 8 (6)	Upper leg	Medium (4 × 12 cm)	Alive
114 (6	13 3 (6	Lower leg	Small (4 × 4 cm)	Dead (31)
16) 3 ɪ 4	16 4 (3 1? 1 (4)	Lower leg	Large	Alive

(a grene of tl e foot developed

Abnormal respiration was noted in 6 of the 10 patients on admission to this clinic (table II) The respiration was rapid and superficial in 4 cases (nos 1, 4, 5, and 7) Artificial respiration was necessary soon after admission in 4 cases (nos 1, 3, 4, and 7) In all, 7 patients were treated in a respirator (nos 1, 3, 4, 5, 7, 8, and 9)

Tachycardia Seven of the 10 patients had pulse-rates above 100 beats per minute on admission to this clinic 2—4 days after the shock

Cyanosis, peripheral coldness, cold perspiration, and pale, slightly yellow skin Marked cyanosis and peripheral coldness before or at the beginning of norepinephrine infusion were noted in cases 1 and 10, data on these symptoms are lacking for the rest of the cases Cold perspiration was noted in 2 patients (cases 1 and 5), but pale, slightly yellow skin was not observed in any of the cases in association with norepinephrine infusion Later, however, all the patients exhibited varying degrees of peripheral coldness, cyanosis, cold perspiration, and pale, yellow skin These symptoms were particularly noticeable in cases 1, 2, and 4

Epinephrine tests

Intracutaneous injection of 0 1 mg of epinephrine by the technique described earlier (14) was given at this clinic to 5 patients (nos 2, 6, 7, 8, and 10) on the 6th, 14th, 7th, 8th, and 2nd day, respectively, after the shock Two of the 5 patients had a characteristic local reaction, "positive epinephrine test" (cases 8 and 10) Later in the disease, repeated tests were negative in these patients, as well as in cases 2, 6, and 7

Intravenous infusion of norepinephrine after admission to this clinic

Norepinephrine was infused intravenously in 2 patients after admission to this clinic One patient (case 1) was given 4 mg during two consecutive days without developing local necrosis In one patient (case 4), in whom blisters had appeared after previous norepinephrine infusion into the left leg, the infusion was continued in the right lower leg, and blisters resembling burns appeared again locally In this case, necrosis also developed in a 49 year old operation scar on the abdomen

Autopsy findings

Six patients died Autopsy showed in all of them a septic spleen, and a swollen liver, in some cases of flaccid consistency and a brownish grey colour, with fat deposits and, microscopically, cloudy swelling Renal cortical necrosis was seen in one case (no 1), as well as necrosis of the liver, spleen, adrenals, and brain In the other 5, the renal cortex was swollen and pale and the marrow was hyperaemic Two cases (nos 4 and 9) showed signs of severe infection, purulent peritonitis and multiple abscesses, respectively Microscopical examination of skin and subcutaneous tissue from the necrotic areas showed, besides the necrotic changes, small haemorrhages and deposits of inflammatory cells

Discussion

Treatment with norepinephrine by intravenous infusion is in most cases carried through without any serious local complications

Kurland and Malach (26) noted no local changes after norepinephrine in fusion in 16 out of 30 patients Necrosis occurred in one case, a 73 year old woman who had cholangitis and septicaemia She received norepinephrine, 4 µg per ml, infused into a vein through a plastic catheter The other 13 developed vascular spasms with local pallor, coldness, and cyanosis and in a few cases vesicular eruptions and resultant superficial ulcerations were seen We noted similar vesicular skin lesions in the marginal zones of the necrotic areas in 2 of our patients (cases 2 and 6) The formation of blisters predominated in one of our cases (no 4)

In 1951 Deterling and Apgar (10) reported the first case with skin necrosis after norepinephrine infusion Extravasation had occurred Cases have been described however, in which obvious extravasation of norepinephrine did not cause skin necrosis (16) Norepinephrine in concentrations up to 20 µg per ml is used in agents for infiltration anaesthesia (32 33, 36) In most solutions for intravenous infusion norepinephrine concentrations higher than 4—8 µg per ml are not used A search through the literature shows that extravasation was noticed in about one fourth of the described cases of skin necrosis after intravenous infusion of norepinephrine In our series extravasation occurred definitely in one case (no 2) and was suspected in one (no 10)

The total amount and the concentration of infused norepinephrine have been considered to play a minor part in the causation of necrosis (4 20) The length of the infusion time seems to be an important factor in the causation of necrosis (3, 4, 20, 29, 30)

Most reports of norepinephrine necrosis (24, 31 41, 46) comprise one or two cases some authors (7, 21, 27 34, 37, 45) have described 4 to 7 cases De Alvarez et al (9) have published detailed reports of 9 cases with severe local complications after intravenous infusion of norepinephrine Vilain and Cubier (43) have presented a brief account of 19 cases Many reports contain no or very sparse clinical data Some anamnestic data are included for about 80 of the cases found in the literature

The shock that necessitated the treatment occurred in 38 % during surgical abdominal diseases, in 16 % during surgical diseases of the chest in 12 % during neurosurgical diseases and in 4 % during other surgical diseases The primary diseases were obstetric gynaecological disorders in 10 % traumatic injuries in 3 % and infectious non surgical diseases in 4 % Myocardial infarction was present i 7 % Shock directly referable to bleeding occurred in 4 %

In none of the published cases of necrosis was there exogenous poisoning for example with barbiturates In about half the cases the norepinephrine solution was infused through a plastic catheter in a peripheral vein

The risk of necrosis is possibly greater in the legs than in the arms (27) Among the published cases of necrosis about 80 % occurred in the lower legs at the site of the infusion In 8 of our 10 cases the necrosis was localized to the lower legs The great saphenous vein is however in many hospitals most commonly used for exposure

Endotoxin from Gram negative bacteria may under special conditions enter the blood stream from the gastro-in

testinal tract as well as from foci of infection in, for instance, the biliary and urinary tracts (11, 22) There is, however, no method available for estimation of endotoxins in blood Most of the published case-reports of norepinephrine necrosis do not allow any conclusions about the presence or absence of endotoxinaemia at the time of the shock The detailed report by De Alvarez et al (9), however, makes the suggestion of endotoxinaemia possible in some cases

Postoperatively, one patient received infected glucose solution intravenously, which produced shock Another tried to induce abortion by introducing a coil spring into the uterus One patient had a blood pressure fall after cholecystectomy

In our cases endotoxin from Gram-negative bacteria may have entered the blood stream in intestinal diseases (4 cases), biliary diseases (3 cases), urinary tract or gynaecological diseases (2 cases), and possibly produced the shock In one patient (case 7) there was no suspicion of endotoxinaemia as a primary causal factor of the shock This was severe and prolonged, however and despite treatment it recurred within a short time Therefore, it may have been possible for endotoxin from the gastro-intestinal tract to enter the blood stream (12)

Experiments on animals (39, 40) and our own studies (13, 14) support the hypothesis that endotoxinaemia is a factor concerned in the causation of norepinephrine necrosis in man We have not, however, been able to analyze all patients admitted to our clinic with tubular necrosis regarding endotoxinaemia and norepinephrine infusion — consequently as yet we have no control material

Our 2 patients in whom norepinephrine infusions were continued after they had been admitted to this clinic are of special interest One of them (case 4) developed not only a new local reaction but also necrotic changes in an operation scar on the abdomen Similar cases with necrotic changes, elsewhere than in the region of infusion, have been described (9) Our other patient (case 1) reacted with local necrosis after norepinephrine infusion, but only in the initial phase of the disease

A patient with "septic toxic" symptoms, who was given infusions of altogether 1,500 mg of norepinephrine over a long period, has been reported to us (6) Local skin necrosis developed only initially Perhaps the conditions for the development of norepinephrine necrosis may be more favourable early in a septic toxic state

Summary

The following factors have earlier been proposed as causes of the skin necrosis that sometimes follows norepinephrine infusions Extravascular infusion, passage of norepinephrine through the vessel wall reflux to smaller vessels, local ischaemia before the norepinephrine infusion, as well as thrombophlebitis and stasis after prolonged infusion We add the proposition that endotoxinaemia may contribute to the developement of such necrosis

Thomas et al found that intracutaneous injection of epinephrine or norepinephrine caused local skin necrosis in

endotoxinaemic rabbits We found in an earlier work that intracutaneous injection of epinephrine caused local bleeding and degeneration of connective tissue fibrils in the skin of some persons This cutaneous reaction was macroscopically characteristic It was seen in 5 % of normal subjects and in about 30 % of our patients with acute renal failure — a disease which in some patients may be due to endotoxinaemia The primary diseases in these patients were cholecystitis peritonitis, septicaemia, colitis, etc Endotoxinaemia was suspected in most of them

Greisman et al found the same cutaneous reaction to epinephrine and norepinephrine in patients with typhoid fever Also from our experiences intracutaneous injection of norepinephrine in man may give rise to the same reaction

As regards rabbits, fever, shock leucocytosis and thrombocytopenia are most regularly seen in provoked endotoxinaemia In our patients referred to above these symptoms were common together with cerebral symptoms (confusion excitation somnolence or coma) and vasoconstriction symptoms (cyanosis peripheral coldness cold sweat and pale slightly yellow skin) abdominal symptoms (diarrhoea intestinal paralysis and intestinal bleeding) and respiratory symptoms (tachypnoea irregular respiration and respiratory failure) It is proposed that in man these symptoms may be part of the clinical picture of endotoxinaemia

In this work we have studied the above mentioned symptoms and other clinical data in the 10 patients with skin necrosis after norepinephrine infusion who appeared at our clinic in 1956—1963 Nine of our 10 patients showed most of these symptoms in connection with a primary disease that was localized to the intestine or to organ that are often the seat of infection with Gram negative bacteria, e g the biliary and urinary tracts The presence of endotoxinaemia in these cases therefore seems possible Endotoxinaemia in such cases cannot be established, however a diagnostic method for estimation of endotoxins is not available

The present work does not prove that endotoxinaemia contributes to the development of local skin necrosis after intravenous norepinephrine infusion in man But the study gives some support to our proposition, here presented as a working hypothesis for further investigations

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From he Eye Cl n c (Head K O Granstrom M D) and the Med cal Cl n c IV (Head S Kallner M D †) Sodersjukhuset Stockholm Sweden

Idiopathic Hypoparathyroidism with Cataract and Spontaneous Hypocalcaemic Hypercalciuria

By

K O GRANSTROM and R HED

Cataract n pat ents w th muscle spasms was described as early as the 18 0 s (6) and the f rst case of cataract n a thyro d ectom zed pat ent w th spasms was re ported n 1880 (6) S nce then great n terest has been focused on such cases It has been poss ble to show that the cause s a d sturbance n calcium metabol sm expressed as hypocalcaem a due to njury to the parathyro d glands at thyro dectomy

In our case on the contrary d opath c hypoparathy o d sm was the cause of cataract It was n fact the appearance f cataract that led to th s d agnos s

Keerl 5 and Lohjala (7 have given ex en e surveys of the l terature on ocular man festat ons n d opath c hypopara hyro d sm to wh ch reference is made

In r pat ent spontaneous hypocal aem hypercalc uria occurred in the c rse of a calc um balance test w thout ne dent exogenous adm n strat on of am n D Th s c rcumstance enabled s t make certa n metabol c stud es

Consequently from both the ophthal mological and the metabol c aspects we cons dered t advisable to report the case n detail

Case report

A 36-year-old woman came for exam nat on n March 1960 because of a success e b lateral decrease in v s on for the past 3 years. Her parents and three s bs had healthy eyes She was said to ha e had some nflam mat n of he eyes as a child and to ha e squin ed sl gh ly but had no other history of eye trouble She had prev ously been heal thy apart f om the usual ch ldhood diseases and had no knowledge of ha ing had rickets or any o her def ciency disease There we e no evident endocrine d s u bances her mens truat on was normal she had not undergone hyro dectomy nor had she had any spasms or con uls ons

Ophthalmological examina ion

V sual acuity 2 60 and 3 60 respecti ely no n provement w th glasses The pressure was somewhat low 11 mm Hg No nflamma tion was present. Apart from a macula of phlyctenular type on the left cornea both corneas were normal In bo h eyes there was

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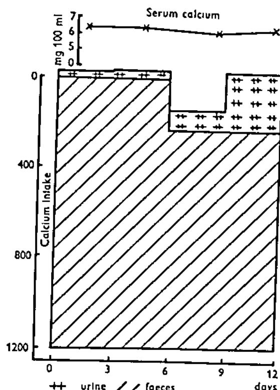


Fig 1 Calcium balance test Intake 1 200 mg/24 hours During the first 6-day period the urinary calcium was low During the later period of the test the calcium in the faeces decreased, with a concurrent increase in the urinary output of calcium During the entire test, the serum calcium was about 6 mg/100 ml

fairly pronounced cataract corresponding to the visual acuity The opacities of the lens were largely radial, and were confined to the outermost layers of the cortex they were more marked in the posterior cortex than in the anterior The fundi were only indistinctly visible, the discs were normal

In view of the appearance of the cataracts and because other aetiological factors were lacking, a disturbance of calcium metabolism was immediately suspected Since the serum calcium was pathologically decreased (5 6 mg/100 ml) the patient was transferred to the Department of Medicine for further investigation

Medical examination

The history was uninformative apart from the patient having been married for 12 years without becoming pregnant although she wanted children Her general condition was good Heart, lungs and abdomen no abnormality found B P 150/80 mm Hg No skin or mucosal lesions Trousseau s and Chvostek s signs were positive No typical signs of pseudo-hypoparathyroidism were present

Routine blood counts were normal The serum calcium ranged from 5 3—6 0 mg/100 ml, and serum phosphorus from 6 2—7 0 mg/100 ml The urinary calcium was about 20 mg/24 hours I v injection of 40 I U of parathyroid extract (Parathormone®) produced a fivefold increase in the urinary output of phosphates There were no signs of impaired renal function The fat in the faeces amounted to 3 4 g/24 hours, and the nitrogen content to 1 2 g/24 hours For the calcium balance, see fig 1

During hospitalization, without exogenous administration of vitamin D, the patient entered a state of hypocalcaemic hypercalciuria It lasted for about 3 weeks, and could be partly recorded in the calcium balance test (fig 1)

Treatment and subsequent course

The patient was treated with vitamin D (Fortedol®), the initial dose was 120 000 I U daily which was later reduced to 60 000 I U daily After institution of therapy the serum and urinary calcium, as well as the serum phosphorus have been normal except soon after childbirth, when the serum calcium fell to 7 6 mg/100 ml on one occasion

After investigation at the Medical Clinic, operation for cataract was performed on the right eye at the Eye Clinic Discission was done first followed twice by evacuation of swollen lens masses after which the residues of the lens were absorbed spontaneously In August 1960 the eye was free from irritation, with a thin secondary cataract, normal fundus and visual acuity of 0 9 with a cataract lens The secondary cataract later became denser and the visual acuity was reduced to 0 1 After new discission in the spring of 1962, the visual acuity was restored to 1 0 (with cataract lens) In the autumn of the same

year the left eye was operated on For some unknown reason, a lengthy state of irritation developed in this eye with loss of light localization and the eye finally had to be enucleated. At the most recent examination (Feb 1964) the right eye was free from irritation and the visual acuity was normal with a cataract lens.

Shortly after idiopathic hypoparathyroidism had been diagnosed and therapy instituted the patient became pregnant. She had a normal parturition on Feb 12., 1961 The child has developed normally and at examination in March 1963 was found to have normal eyes without cataract.

Discussion

Ophthalmological aspects

It has been shown that, in adults cataract associated with tetany is caused by hypocalcaemia Zonular cataract in children has a similar pathogenesis The experimental studies of von Bahr (2) among others have established that zonular cataract is due to disturbances in calcium metabolism leading to hypocalcaemia and tetany

The characteristic feature of a cataract due to hypocalcaemia is that the opacity lies in the outermost zone of the lens If the disturbance ceases and the lens is still growing a typical zonular cataract arises where a clear lens zone lies beyond the opaque one Occasionally two successive opaque zones can be seen indicating disturbances in calcium metabolism at two different periods In experimental animals it has even been possible to obtain several different opaque zones Consequently in zonular cataract in children the situation of the opaque zone permits conclusions regarding the time at which the disorder of calcium metabolism occurred It the zonular cataract is small, the disturbance must have been an early one — possibly already during foetal development — whereas if the cataract is larger, it must have occurred relatively late

This can be illustrated by two cases in in which tetany due to hypoparathyroidism was definitely responsible for cataract (3) One was a young girl, in whom symptoms of tetany appeared after thyroidectomy at 12 years of age Visual disturbances subsequently appeared, and she came for examination at 19 years of age She then had a pronounced unusually large zonular cataract, with an extremely thin layer of clear lens substance beyond the opaque zone Thus, growth of the lens had not ceased at the time of tetany She was operated on, and good visual acuity was achieved with cataract lenses for both eyes The other patient was a woman in whom thyroidectomy at 16 years of age was followed by severe tetany At the age of 32 she exhibited severe cataract with subcapsular opacities but no zonular cataract The growth of the lens was therefore essentially completed at the time when she had tetany

In this connexion mention can be made of two cases of zonular cataract in children of diabetic mothers described by one of us 4 These cases seem to be of great interest since they may be an indication of some disturbance of calcium metabolism in the diabetic mother which led to zonular cataract in the foetus

Medical aspects

The investigations made in our case could rule out renal damage and a

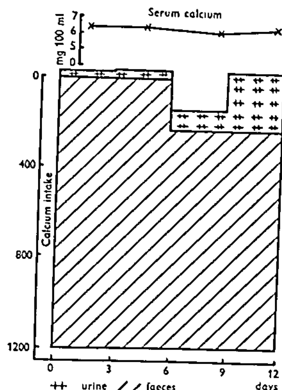


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hypoparathyroidism whose pregnancy went to term

After parturition, our patient's serum calcium fell to 7 6 mg/100 ml on one occasion This stresses the importance of keeping an extra careful check on the calcium metabolism of such patients during the later part of pregnancy and during lactation

Summary

An account is given of a case of idiopathic hypoparathyroidism in which the appearance of cataract led to the diagnosis

During hospitalization a state of hypocalcaemic hypercalciuria occurred without exogenous administration of vitamin D The calcium balance test showed that this was associated with an increased resorption of calcium from the intestine The possibility is discussed of a transient vitamin D effect, brought about by intense solar irradiation

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disturbance of resorption as the cause of hypocalcaemia Consequently, some form of parathyroid insufficiency must be present The blood and urinary findings, consisting of low serum and urinary calcium and high serum phosphorus, are best compatible with the existence of hypoparathyroidism

Since no operative intervention had been made on the neck, nor any radioactive iodine treatment given, it can only be a question of idiopathic or pseudohypoparathyroidism The patient lacked any signs typical of pseudo hypoparathyroidism, and i v injection of parathyroid extract produced a prompt reaction, in the form of considerable phosphaturia Consequently, the diagnosis of idiopathic hypoparathyroidism must be regarded as established

An interesting feature from the metabolic point of view is that the patient spontaneously, without exogenous vitamin D administration, entered a state of hypocalcaemic hypercalciuria Since the reversal from hypo- to hypercalciuria occurred in the middle of a calcium balance test, we were able to make certain metabolic studies (fig 1) During the first two 3-day periods of the test, only small quantities of calcium were present in the urine During the later periods, on the contrary, a considerable increase in urinary calcium occurred (output 308 mg/24 hours), but without a rise in serum calcium A coincident, corresponding decrease in the faecal excretion of calcium was recorded The hypercalciuria ceased after 2—3 weeks

Such states of hypocalcaemic hypercalciuria in hypoparathyroid patients do as a rule, occur only in connexion with vitamin D therapy, particularly if the diet contains plentiful cheese and milk

Albright and Reifenstein (1) did, however, report three cases of hypoparathyroidism with spontaneous hypocalcaemic hypercalciuria In one of them, the cause was thought to be a transient urinary tract infection They were unable to give any explanation in the other two cases

In our case, no urinary tract infection was present During hospitalization, the patient sat in the sun for several hours every day, so that she was strongly sunburnt It should perhaps be pointed out that this exposure to the sun occurred in the spring, directly after the dark winter season In our opinion, it is not improbable that the hypocalcaemic hypercalciuria in this case can be interpreted as a transient vitamin D effect, caused by the solar irradiation This view is supported by the increased resorption of calcium from the intestine, recorded in the calcium balance test Moreover, we do not consider it unlikely that some of the cases of spontaneous hypocalcaemic hypercalciuria described in the literature can be explained in the same way

Another feature which is perhaps noteworthy is that the patient's 12 year marriage had not resulted in pregnancy despite her desire to have children As soon as an adequate dose of vitamin D had been prescribed she became pregnant Obviously it cannot be stated with certainty whether hypoparathyroidism had been of any consequence for her involuntary sterility but this appears likely The literature does however contain reports of patients with untreated

hypoparathyroidism whose *pregnancy went to term*

After parturition, our patient s serum calcium *fell* to 7 6 *mg/100* ml on one occasion This stresses the importance of keeping an extra careful check on the calcium metabolism of such patients during the later part of pregnancy and during lactation

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Acute Gouty Arthritis Provoked by Cerebrovascular Disease

By

Ole Evald Hansen

Acute gouty arthritis often occurs in connection with trauma operation infection allergic reaction exposure to cold or heat and following mental stress (27). Attacks after the administration of certain drugs have also been described for example therapeutic doses of pyrazinamide (9 36) and chlorothiazide (8 10).

According to Gutman et al (18) the pathogenesis of secondary gouty arthritis differs from that of primary gouty arthritis the disease being defined as a complication chiefly in haemopoietic diseases characterized by increased nucleic acid metabolism In some cases gouty arthritis may be of secondary occurrence to such diseases which are often accompanied by hyperuricaemia

In addition gouty arthritis has been observed in a few cases following myocardial infarction Cohen (6) for example observed acute gouty arthritis in two patients after an attack of acute myocardial infarction at an interval of 36 hours and 6 days respectively, and Ask Upmark and Adner (2) reported three cases of gouty arthritis following the same disease

During the last decade, several studies have been published on the relationship between hyperuricaemia and degenerative vascular disease (19), but only few reports are available dealing with hyperuricaemia and cerebrovascular disease (20 21) Gouty arthritis does not appear to have been mentioned previously in connection with these diseases The following 5 case histories would seem to support the theory that a connection exists between cerebrovascular disease and hyperuricaemia

Case reports

Case 1 64 year-old brewery worker Over a period of 4—5 years prior to hospitalization on 26 VIII 1961 the patient had repeated attacks of gouty arthritis localized mainly to the metatarsophalangeal joint of a great toe Other joints were also attacked for example the right carpus 14 days prior to admission to hospital There had been palpitations for

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Case 2 77 year-old retired manufacturer, who had had a transient left sided hemiparesis 18 months prior to hospitalization on the 28 II 1963 He had suffered from gout for some years prior to admission On the 25 II the patient woke up with paralysis of the right upper extremity, and paraesthesia of both lower extremities Physical examination showed that his general condition was unaffected The patient was conscious but only partly lucid There was a suggestion of a Parkinson mask Apart from bilateral impairment of hearing there was no paresis of the cranial nerves There was flaccid paralysis of the right upper limb The right hand was in the pyramidal position and with almost continuous myoclonus Deep tendon reflexes were missing in the lower extremities plantar reflexes were normal Gait was straddling slightly atactic Ophthalmoscopy retinal arteriosclerosis slight anisocoria (R l synchysis scintillans of the right eye subacute conjunctivitis BP 165/80 weight 83 4 kg height 164 cm ESR 20 mm/h Hb 153 g/l WR negative lumbar puncture *normal pressure and cell count* Serum creatinine 20 mg/l urine negative sugar and protein

ECG normal Roentgenogram of thorax long standing pulmonary tuberculosis sequelae of bilateral pleurisy slightly enlarged heart Roentgenogram of cranium halisteresis

EEG severe abnormality with 1 1/2—3 *c/s activity in left temporal area*

On the 3 III i e 6 days after the cerebrovascular insult there was a violent erythema and tenderness of the metatarsophalangeal joint of the left great toe Serum uric acid 59 mg/l (two days previously 73 mg/l) The patient was treated with phenylbutazone and the joint discomfort gradually disappeared On discharge from hospital there was persistent paralysis of the right hand but the patient could raise his right arm to the *horizontal*

The patient was admitted moribund to medical *department* C Copenhagen *County* Hospital Glostrup on the 14 IV 1963 dying about 5 hours later Autopsy diagnoses Infarctus *recent parietis posterior* cordis, infarctus vetus parietis ant septi cordis thrombosis a coronar dx hypertrofia cordis in primo ventriculi sin, arteriosclerosis aortae et a coronar m gr, stasis chronica hepatis, pleuritis fibrosa et fibrinosa, peritonitis fibrinosa et fibrosa adhæsiva, stasis et oedema *pulmonum nephrosclerosis*

Comments

77 year old retired manufacturer with transient left sided hemiparesis for 18 months prior to admission and gout 2 years prior to admission Three days prior to admission the patient woke up with paralysis of the right upper limb and paraesthesia of the lower extremities The very elective attack with motor involvement of the arm, together with a well defined EEG focus, favoured a small cortical infarct rather than a brain stem infarct The diagnosis of cerebral infarction must be considered clinically absolutely certain Six days after the attack the patient developed gouty arthritis in the metatarsophalangeal joint of the left great toe Serum uric acid was 59 *mg/l*, but two days previously it had been 73 mg/l About 4 weeks after discharge the patient was admitted to the medical department where he died some 5 hours later Autopsy showed a fresh infarction of the posterior wall of the myocardium and severe arteriosclerosis of the aorta and the coronary arteries

Case 3 83-year-old retired director who had suffered from attacks *of gyratory vertigo* for 8—10 years In 1945 the patient had *undergone an operation for stone in the* bladder Twenty four hours prior to admission on 21 V 1963 he became acutely ill with feelings of numbness in and paralysis of his left upper extremity There was no loss

approximately 5 years Speech disturbances developed suddenly after work on the day of admission to hospital There were no pareses

On admission to hospital, physical examination showed an adipose pyknic, reacting adequately to simple requests, but able to say only yes and no Ophthalmoscopy retinal arteriosclerosis and grade I hypertonic fundus There were no other signs of organic nervous disease Heart rhythm irregular BP 150/100—215/130—180/120—160/85 Weight 78 5 kg, height 165 cm ESR 6 mm/h, Hb 154 g/l, WR negative Urine negative sugar, positive protein Serum creatinine 13 mg/l Serum uric acid 88 mg/l (28 VIII) and 77 mg/l (8 IX), serum cholesterol 2 8 g/l

ECG Left sided axis deviation, atrial fibrillation Roentgenogram of thorax showed heart 16 5, thorax 31 cm The aortic arch was prominent and showed calcifications

EEG Moderately severe abnormality, 3—4 c/s complexes in the left temporal region with spread to the remaining left sided leads

On the 30 VIII, arteriography was performed in the left common carotid artery under anaesthesia The examination showed a filling defect of the anterior cerebral artery, and pronounced arteriosclerosis of the siphon and of the internal carotid artery on the neck, where considerable irregular stenosis was seen just beyond the point of bifurcation There was good filling if the anterior arteries and of the middle cerebral artery There was pronounced arteriosclerosis at the level of the siphon Just beyond the point of bifurcation there was pronounced, irregular stenosis of the internal carotid artery

On the 2 IX the patient was transferred to the neurosurgical department, where the intention was to perform a vascular operation but this was given up in view of the patient s poor cardiac condition

On the 7 IX, i e 12 days after the cerebrovascular attack, there was erythema of the skin over the left patella, where there was fluctuation over the bursa præpatellaris During the next few days the temperature rose to a maximum of 38 2° C

On the 8 IX, ther was pain, erythema, swelling and tenderness of the right medial forefoot, particularly round the great toe The patient received treatment with cinchophen with good effect, but as a result of vomiting, treatment was changed to phenylbutazone The patient was discharged from medical department B on digitalis and probenecid therapy

On the 11 IV 1962, the patient was operated on for hydrocoele of the left testis under local anaesthesia He was discharged from hospital on the 16 IV, and readmitted 10 days after operation with the diagnosis of superficial phlebitis of the right foot, which was swollen and hot The discomfort soon disappeared following treatment with phenylbutazone This may have been a question of gouty arthritis

Comments

64-year-old brewery worker with palpitations and attacks of gouty arthritis for 4—5 years, the last attack being in the right carpus a fortnight prior to an acute cerebral infarction, with aphasia Bilateral carotid arteriography showed pronounced arteriosclerosis of the internal carotid arteries The patient was transferred to the neurosurgical department for vascular operation, but this was abandoned owing to poor cardiac condition Twelve days after the acute cerebral insult the patient developed acute gouty arthritis in the left prepatellar bursa, and the next day in the right great toe Hyperuricaemia had been demonstrated at routine examination 10 days previously Seven to eight months later the patient was operated on for hydrocoele of the testis, and he was readmitted for superficial phlebitis of the left foot 10 days after this operation It is probable that postoperative gouty arthritis had been involved

TABLE I Review of case history and serum uric acid in 5 males with acute gouty athritis (g a) following cerebral infarction The uric acid determinations were carried out as enzymatic spectrophotometry by Prætorius method in which the extreme values for males are 26—75 mg/l (16)

Patient no	Previous acute g a	Previous symptoms from stone in the urinary tract	Serum uric acid mg/l on admission in acute g a		Interval between infarction and acute g a
1	+	0	88	77	12 days
2	+	0	73	59	6 days
3	0	+	84 5	84	5 days
4	+	+	96	96	12 hours
5	+	0	71	not measured	5 days

acute left hemisphere infarction Immediately after admission he developed gout and hyperuricaemia was demonstrated Severe right sided hemiparesis was still present on discharge

Case 5 56 year-old dairy proprietor From his 28th to his 45th year the patient had had frequent painful joint attacks with erythema and swelling of the joints of the foot great toes and fingers *alternately From* about 1956 the patient had had regular probenecid treatment which led to the disappearance of the joint trouble During the last two years the patient has had difficulty in managing his shop because of a poor memory In 1958 diabetes mellitus was diagnosed for which he was prescribed dietary treatment In 1962 the patient was admitted to hospital for a cerebrovascular insult without permanent sequelae The present illness started suddenly on the day prior to admission on the 2 VIII 1964 when there was paresis of the left foot and arm Physical examination showed adiposity slight dyspnoea during examination Both knee joints were slightly swollen there was a slight diffuse impairment in strength in the left upper extremity but normal reflexes and sensitivity Slight diffuse impairment in the strength of the left lower extremity and left sided Babinski

Weight 84 8 kg height 171 cm BP 210/110—150/95—140/80, ESR 16 mm/h Hb 159 g/l WR negative 24 hours excretion of sugar 5 g Blood sugar 182 — 161 — 128 mg % on 1 200 calories Roentgenogram of cranium central calcified pineal body ECG normal EEG normal Ophthalmoscopy fundus hypertonic in grade I—II Lumbar puncture pressure 180 mm H_2O clear and colourless fluid 0/3 leucocytes and 205/3 erythrocytes protein 284 mg/l Intelligence test showed organic reduction

On the 6 VIII i e 5 days after the vascular insult there was pain erythema and swelling of the left bursa olecrani and upper part of the left dorsum antebrachii On the 3 VIII the serum uric acid value was 71 mg/l and the serum creatinine value 11 mg/l On the 15 VIII the serum uric acid value was 57 mg/l after 8 days treatment with probenecid Neurological examination four days after admission showed normal conditions

Comments

56 year old dairy owner with numerous attacks of gouty arthritis from the age of 28 to the age of 45 The joint discomfort disappeared as a result of continued probenecid treatment Six years prior to the present admission, a mild dia

of consciousness Physical examination showed clear mental state, alert, well preserved Slight diffuse reduction in strength in the left upper extremity, with a positive Barré test A normal sense of joint position and number sense No definite paralysis of the lower extremities, but spreading of the toes of the left foot on plantar reflex Ophthalmoscopy incipient cataract of both eyes, synechia of the pupils, sequelae of retinal thrombosis of the right eye EEG border case with slightly reduced dominant frequency generally Roentgenogram of the cranium arteriosclerosis of the internal carotid artery Roentgenogram of the thorax heart increased in width, pulmonary congestion and arteriosclerosis of the aorta Lumbar puncture pressure 180 mm H_2O, clear and colourless fluid, 1/3 leucocytes and 3/3 erythrocytes, 504 mg/l protein ECG normal BP 170/90, ESR 41 mm/h Hb 151 g/l, urine negative protein and sugar, serum creatinine value 14 mg/l Weight 84 5 kg, height 170 cm

On the 25 V, i e 5 days after the cerebral attack the patient developed pain in the metatarsophalangeal joint of the left great toe, and the right tuberosity of the tibia The skin was reddened at the sites in question, and there was a moderate swelling and acute tenderness of the metatarsophalangeal joint of the left great toe These discomforts disappeared following treatment with oxyphenbutazone (Tanderil) Serum uric acid was 84 5 mg/l on admission and 84 0 mg/l at the onset of the gouty arthritis

Comments

83-year old retired director, admitted to hospital with acute cerebral infarction Hyperuricaemia was found on admission and 5 days later during an acute attack of gouty arthritis

Case 4 70 year old store manager Conservative treatment in 1961 for stone in both kidneys For 6 months prior to admission to hospital on 3 II 1964 the patient had had nagging pain nightly in the right great toe, and had previously been under treatment for hypertension, but not during the last two years On the day of admission, the patient woke up with paralysis of the right sided extremities No impairment of consciousness, no vomiting nausea, or vertigo Physical examination showed the patient to be mentally clear and well oriented, with a massive right sided lower facial palsy The right upper extremity showed paralysis and absence of tendon reflexes The tendon reflexes of the left upper extremity were weak The right lower extremity was site of a moderately severe diffuse paralysis, and there was a right sided Babinski reflex Opthalmoscopy hypertonic fundus of grade II + retinal arteriosclerosis Lumbar puncture pressure 100 mm H_2O, clear and colourless fluid, 2/3 leucocytes and 10/3 erythrocytes, 348 mg/l protein. BP 160/80, weight 71 4 kg, height 174 cm ESR 18 mm/h, Hb 110 g/l — 147 g/l ECG left sided axis deviation, depression of ST_I and ST_{II}, neg T_I Chest leads moderate left sided ventricular hypertrophy

EEG moderately severe abnormality 1 1/2—3 c/s activity of slightly increased amplitude bitemporally with alternating but most often left sided preponderance Dominant activity generally reduced Left sided carotid arteriography showed arteriosclerotic plaques immediately beyond the bifurcation Roentgenogram of thorax normal

The following values were found serum cholesterol 2 5 g/l, serum uric acid 96 mg/l (4 II) serum creatinine 14 mg/l Urine was negative for protein and sugar Immediately after admission the patient complained of pain around the metatarsophalangeal joint of the right great toe On discharge from hospital there was still a fairly pronounced right sided hemiparesis

Comments

70-year-old retired store manager with previously demonstrated stone in both kidneys Periodic nocturnal pain in the right great toe for 6 months The patient was admitted to hospital for an

mined 21 medical *students immediately* prior to appearing for their final examination These authors found no pronounced *shift in the* concentration of serum uric acid in comparison with the values obtained *48 hours later*

Finally it might be worth considering whether hyperuricaemia could be provoked by loss of tissue caused by the cerebral infarction Along with certain other provocative factors this might result in an increase in the *concentration* of uric acid in the serum Seegmiller and Howell (35) favour the idea that even a *modest increase* in the serum uric acid in hyperuricaemic patients might lead to an increased formation of uric acid crystals which again might result in an acute attack in such patients There is not the slightest doubt however, that hyperuricaemia in itself is insufficient to provoke gout Other factors must be present to provoke this disease In agreement with this, Hench (25) and Gutman and Yu (17) express doubt that uric acid should be able to cause local changes Their view gains weight from the fact that administered intravenously uric acid cannot provoke an acute attack either in patients with gouty arthritis or in normal individuals and colchicine which has no effect on the synthesis or excretion of uric acid has a good effect on the acute attack It may be mentioned finally that probenecid — which is without any effect during attacks — will increase the excretion of uric acid

In the five patients in question the joint symptoms appear to have been provoked in some manner by the cerebrovascular disease

Hyperuricaemia has been found in degenerative vascular diseases, the clinical impression that a relationship exists between gout and ischaemic heart diseases (28, 32, 33, 34) has found support mainly in the investigations by Gertler et al in 1951 (14) Since then, several studies have appeared on this topic (see 19) An *attractive working* hypothesis would be to regard the two diseases in the above mentioned patients, viz gout and *cerebral infarction,* as manifestations of the same basic *disease* In the course of time, a *number* of studies have been published in support of the hypothesis that uric acid is involved in atherogenesis Hyperuricaemia occurs frequently in essential hypercholesterolaemia (1, 22), and hypercholesterolaemia is no rare occurrence in gout (3 7, 13) In addition, aspirin, which is a well known uricosuric agent, also affects the cholesterol metabolism, as demonstrated by Eidlitz (12) This author administered a daily dose of 1 5 g of aspirin to 10 patients with degenerative vascular disease for a period of up to one month There was a fall in the blood cholesterol in all 10 patients, and a decrea e in the uric acid concentration in 6 of the patients The *author* concluded that a metabolic relationship must exist between serum cholesterol and uric acid

Hyperuricaemia has also been described in connection with cerebrovascular diseases The present author (21) found hyperuricaemia in 41 (36 %) out of 115 unselected patients with cerebral infarction and in a previous investigation (20) on a consecutive series of 41 apoplectic patients, 12 of them were

betes mellitus was found, and the patient has been on a diet since Two years prior to admission there was a cerebrovascular insult with rapid remission The patient was hospitalized for a sudden, slight, cerebral infarction of uncertain localization Five days later, there was erythema and swelling of the left bursa olecrani The neurological symptoms showed rapid remission

Discussion

The interval of hours or days which elapses from the occurrence of a cerebral infarction to the onset of gouty arthritis might well be accepted without further consideration as expressing an allergic manifestation The hypothesis of allergic genesis was in fact also ventilated by Ask-Upmark and Adner (2), when they described some cases of gouty arthritis, which became manifest 5—12 days after the myocardial infarction In support of this theory, the authors point out that the interval between precipitating factor and joint symptoms is substantially the same as that observed in allergic manifestations such as serum disease, for example Adlersberg (1) stresses that the sudden onset of joint symptoms, as well as the return to normal, both tell in favour of the allergic genesis of acute gouty arthritis

A feature common to the different provocative factors is the primary increase in the adrenocorticotrophic hormone, followed by a reduced secretion of this hormone Hellman's mention of ACTH-provocation of an acute attack in two out of four patients suffering from gouty arthritis is of interest in this connection The attacks occurred on the 3rd or 4th day after the injection (23)

Apart from certain points of similarity to allergic manifestations, for example the symptom free interval, there is no certain and decisive evidence, however, that acute gouty arthritis should have an allergic or endocrine aetiology (29, 42)

It was mentioned in the introduction that gouty arthritis may be provoked by surgical intervention Hench and Darnall (26) called attention to postoperative gouty arthritis, by means of their axiomatic generalization "to suspect gout in cases of acute postoperative arthritis, especially in males", but as early as 1889, Dukworth (quoted in 38) observed that "fits of gout may be brought on by operation" Talbott (38), reviewing case histories from 18 patients with untreated gouty arthritis, found a postoperative incidence of gouty arthritis of 86 per cent after 34 operations In the first case history of the present study, the acute joint attack was preceded by both cerebrovascular disease and bilateral carotid arteriography carried out under anaesthesia, and it is not possible to establish which of these factors provoked the gouty arthritis In the remaining four patients, the joint symptoms and the other manifestations of acute gout were not preceded by angiography, and the temporal relationship between the cerebral infarction and the gouty arthritis may be taken as supporting a causal association

A change in the uric acid concentration could be provoked by the patient being placed in a situation of mental stress Dreyfuss and Czaczkes (11) exa-

history of gouty arthritis was available in four of the patients, and of stone in the bladder in the fifth patient. The possibility is discussed that the two diseases may be manifestations of the same basic disease and the frequent occurrence of hyperuricaemia in degenerative vascular diseases as well as the uricosuric action of anticoagulants are emphasized

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found to have hyperuricaemia (29 %). These figures clearly suggest a relationship between the two diseases.

The high mortality due to coronary infarction found in Finland (4), where gout is a rare disease (5), seems to tell against the atherogenic effect of uric acid. Nevertheless, this objection can be dismissed. Although hyperuricaemia is a cardinal sign in gout it may be the sole manifestation (43). It is estimated to occur from 5 (40) to 10 (31) times more frequently than symptomatic gouty arthritis, or in other words, only about 20 % of the patients with hyperuricaemia will develop articular symptoms (31).

Gertler et al. (15) have discussed the hypothesis that uric acid is a factor of significance in the deposition of cholesterol in the intima. Smythe et al. (37), in isotope studies, showed that patients with gout have increased bone marrow activity in the form of blood platelet turnover, as well as a greater platelet adhesiveness and plasma thrombin activity, than have a control group of patients without gout and atherosclerotic diseases.

Traut et al.'s report (41) on specific vascular lesions in two patients dying from gout provides further support for the hypothesis of the atherogenic action of uric acid. In one of the patients, urate was found in the proliferating intima, and in the other patient, urate deposits were found in the heart musculature, in addition to the nonspecific arteriosclerotic lesions of gout. These cases are of further interest, as they represent the only cases of gout in the autopsy records of the Presbyterian Hospital and Cook County Hospital, Chicago. During the period 1.I.1945 to 15.IX.1952, 10036 autopsies were performed in the latter hospital, and only one of these autopsies was recorded as gout.

This can hardly be attributed to any rare occurrence of gout, but rather to a lack of interest in the disease. Hench and Darnall (26) point out that on the basis of the interest exhibited in the medical literature, this disease seems to have been neglected. During a period of 15 years, up to 1933, an average of two articles annually have been published in English on this topic. During subsequent years, the lack of interest is emphasized by the appearance of articles with titles like "gout, a forgotten disease", and "gout, still a forgotten disease", in 1936 and 1946, respectively (24, 30). Talbott (39) remarks in 1957 that gout as a topic was forgotten or dropped at the beginning of this century, but that it has again come into fashion, and "it may appear in any physician's practice", as the first case history here emphasizes.

None of the 5 patients mentioned received anticoagulation treatment prior to the joint symptoms which accompanied the cerebral infarction. The potent uricosuric effect which has been demonstrated in several of the anticoagulants used (see 19) would therefore appear to be of more than theoretical interest, as early anticoagulant therapy of gouty patients with acute cerebral infarction might prevent articular symptoms.

Summary

Five cases are described of acute gouty arthritis which occurred in close association with acute cerebral infarction. A

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Lipids, Lipoproteins and Proteins in Serum following Partial Gastrectomy

By

KNUT KIRKEBY

Total as well as partial gastrectomy lead to nutritional disturbances caused by defective digestion and absorption of the food particularly of fats and proteins Although a severe degree of steatorrhea relatively seldom follows partial gastrectomy a minor or moderate defect in the assimilation of fat may be demonstrated in the majority of patients A critical review of metabolism studies after subtotal gastrectomy showed that a defect in the assimilation of fat was demonstrated in two thirds of the individuals (3) Steatorrhea was found in patients with severe digestive symptoms as well as in those whose sympoms were mild or absent and the increase in fecal fat was the same in both groups The disturbance of fat digestion is found mainly following the Billroth II operations and patients with a gastro-duodenal anastomosis usually reveal a normal or nearly normal fat digestion and absorption 22 4 11 17) The high frequency of mild or moderate steatorrhea in patients with a gastrojejunostomy has been confirmed by isotope studies (17 20, 14 19) In one rather extensive study abnormal loss of fat in the stool was demonstrated in 75 per cent of the patients (14)

A disturbance in absorption apparently occurs less often for protein than for fat In the reveiw by Everson an increase in fecal nitrogen (above 2 grams of nitrogen excreted) was found in one fourth of the patients (3) Usually the nitrogen loss is not marked and even after total gastrectomy a very high fecal nitrogen is found only occasionally (13)

It has been suggested that the impairment of fat absorption in these patients could be reflected in a lower incidence of coronary heart disease (21) If this was true it might be expected that the gastrectomy patients would reveal subnormal values of some lipid and lipoprotein fractions

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described by Grassmann & Hannig (5 6) The staining was done with Amido black 10 B and the reading with an automatic scanning device The values in g/100 ml were calculated from the *total serum proteins* determined by a biuret method (10) standardized with a micro-Kjeldahl analysis (7)

All lipid analyses have been done in duplicate and average values taken

Results

The subjects in the partial gastrectomy group were on the average of lower weight than those of the control group and the average weight/height relationship evaluated by Broca s formula was — 5 and — 1 respectively in the two groups

When a classification of the participants into thin medium and fat was made by defining the medium class as subjects with Broca values from — 5 to — 5 a difference in distribution of the subjects of the two groups between the fatness classes was apparent A chi square test revealed this difference to be significant ($p < 0\ 05$)

The results of the determinations of serum lipids and lipoproteins are recorded in table I The mean values of cholesterol phospholipids C/P ratio and beta lipoproteins in mg/100 ml of the two groups did not differ The total lipids were slightly higher in the gastrectomy group but the difference between the two means 911 mg/100 ml and 847 mg 100 ml in the gastrectomy and control group respectively, was not statistically significant when evaluated by a t test ($0\ 10 > p > 0\ 05$)

However a small difference between the groups was apparent in the ratio of beta to alpha lipoproteins with 77 6 per cent of beta lipoproteins on the average in the gastrectomy group as against 80 4 per cent in the control group ($p < 0\ 05$)

The results of the serum protein studies are shown in table II There was no difference between the means of total protein determinations in the two groups By paper electrophoresis of proteins a slightly higher value for albumin was found in the gastrectomy group ($p < 0\ 05$) whereas no difference in globulin fractions were demonstrated

TABLE II Serum proteins in normal and gastrectomized men Mean values with S D in brackets

Serum proteins (g/100 ml)	Control material	Gastrectomy patients
Albumin	3 57 (0 28)	3 72 (0 33)
α_1 globulin	0 39 (0 08)	0 34 (0 08)
α_2-globulin	0 61 (0 09)	0 61 (0 13)
β-globulin	0 93 (0 15)	0.93 (0 16)
globulin	1 47 (0 24)	1.38 (0 23)
Total	6 97 (0 38)	6 98 (0 44)

Comments

If disturbances of fat absorption following partial gastrectomy had resulted in a significant modification of the lipid metabolism, one might have expected a lowering in the serum concentration of cholesterol phospholipids and beta lipoproteins as well as of C/P ratio No such changes have been demonstrated The findings therefore do not substantiate the theory that partial gastrectomy may

TABLE I Serum lipids and lipoproteins in normal and gastrectomized men Mean values with S D in brackets

	Control material	Gastrectomy patients
Cholesterol (mg/100 ml)	255 (45)	244 (48)
Lipid phosphorus (mg P/100 ml)	9 9 (1 8)	9 6 (1 5)
C/P ratio	26 1 (4 7)	25 8 (5 8)
β lipoproteins (mg/100 ml)	684 (141)	708 (163)
β lipoproteins (%)	80 4 (7 6)	77 6 (5 9)
Total lipids (mg/100 ml)	847 (137)	911 (181)

The object of the present study has been to study the effects of partial gastrectomy on the serum levels of lipids, lipoproteins and proteins

Material and methods

The material comprises 51 patients who had a partial gastrectomy performed and a control material of 47 healthy subjects The individuals of both groups were men from 35 to 55 years of age The average ages of the two groups did not differ significantly

The *control material* included hospital personel and employees and workers from a factory The examination of this group included family history personal history physical examination blood pressure reading examination of the urine for sugar and protein, sedimentation rate ad modum Westergren hemoglobin determination and measurement of height and weight An electrocardiogram with standard leads and unipolar extremity and precordial leads was also recorded Only persons without history or signs of major disease were included in the study Everyone declared themselves to be in good health and without recent illness of any kind The range of hemoglobin determinations was from 13 2 g/100 ml to 16 0 g/100 ml Subjects with a blood pressure higher than 140/90, a sedimentation rate higher than 15 mm in one hour, pathologic changes in the electrocardiogram or a history of recent weight change were rejected from the study

The range of weight/height relationship according to Broca's formula (weight, kg — (height, cm — 100)) in the control material was from + 10 to — 15

The gastrectomy group had their operation performed 5 to 10 years prior to the investigation The operation was a Billroth II procedure with gastro jejunal anastomosis This group had the same general examination as the control material

Total serum cholesterol was determined according to a modification of the method of Kingsley & Schaffert (9)

The determination of *lipid phosphorus* was carried out by evaporation of an ethanol ether filtrate to dryness in a water bath, followed by wet ashing of the filtrate with a one-to one mixture of conc HNO_3 and $HClO_4$ Inorganic phosphorus was determined according to Berenblum & Chain s method as modified by Martin & Doty (1, 12)

The cholesterol/phospholipid ratio (C/P ratio) has been calculated as

$$\frac{\text{mg cholesterol 100 ml}}{\text{mg P/100 ml}}$$

The serum lipoproteins was determined by paper electrophoresis according to a modification of the method of Jencks et al (8) The results have been recorded as per cent of total lipids and mg/100 ml of beta lipoproteins

The procedure for *total lipid* determinations was a modification of the method described by Swahn (18)

The paper electrophoresis of proteins has been performed with a modification of the method

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result in changes of the serum lipids similar to those observed on a low fat diet

It is evident that the total amount of intestinal fat assimilation is dependent not only upon the per cent of fat absorbed An increase of ingested fat leads to a proportional increase of absorbed fat also in gastrectomy patients (15) It has been stated that these patients actually consume more fat than is common in the general population, and a high fat diet has been advocated as a treatment for digestive distress following gastrectomy (2) It is possible that some patients discover the relieving effect of a high fat diet and thereby also compensate for the disturbance in fat absorption However, the question as to whether or not a preference for high fat diet actually is the cause of the normal serum cholesterol after gastrectomy cannot be answered without an exact quantitative dietary survey

The investigation has revealed a small, but statistically significant difference in the ratio of beta to alpha lipoproteins — with a higher per cent of beta lipoproteins in the control group The meaning of this finding is obscure The possibility that gastrectomy may influence lipid metabolism in other respects than via fat absorption is suggested by the demonstration of a heparin like effect of gastric mucin (16) The present study did not include determination of triglycerides The difference between the means for total lipids, 911 mg/100 ml and 847 mg/100 ml, in the gastrectomy and control group respectively, was close to statistical significance ($p < 0.10$), and might possibly indicate a difference in triglycerides, since mean values for cholesterol and phospholipids were of the same size in the two groups.

The study of serum proteins did not indicate any protein deficit in the gastrectomized patients In fact, a slightly higher mean value for serum albumin was noted in this group This might be related to the difference in beta- to alpha lipoprotein ratio, since a large part of the alpha lipoprotein fraction is located within the albumin region in the paper electrophoresis

Summary

Studies of serum lipids, lipoproteins and proteins have been performed in 47 normal and 51 partially gastrectomized men in the age group 35 to 55 years

No significant difference between the two groups was demonstrated with regard to total cholesterol, lipid phosphorus, C/P ratio, or beta lipoproteins in mg/100 ml From these findings it might be concluded that the moderate impairment of fat absorption usually found in gastrectomized patients does not result in modifications of lipid metabolism comparable to those seen on a low fat diet The mean value obtained for total lipids in the gastrectomy group was higher than that for the control group The difference between the means was close to statistical significance and the possibility of a difference in triglyceride levels between the two groups is discussed

The study of serum proteins did not indicate any protein deficit in the gastrectomized patients

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The Kinetics of Bromsulphalein Elimination during Continuous Infusion in Man

By

K Winkler and C Gram[1]

When bromsulphalein is given as a continuous infusion the concentration in the arterial blood may either rise constantly or eventually reach a plateau For linearly rising concentration the maximal capacity of the liver for removal of bromsulphalein has been calculated by several authors When the concentration is constant the plasma clearance of bromsulphalein calculated from infusion experiments with constant concentrations has been used as a measure of liver function

As both the maximal removal capacity and the plasma clearance would be of great importance as quantitative measures of the excretory function of the liver the assumptions and validity underlying these calculations have been studied further The results obtained have been compared with a model of the elimination of bromsulphalein which previously has been discussed from similar studies with single intravenous injections of bromsulphalein 20)

Material and methods

The examinations were made on patients without signs of liver disease and circulatory disturbance They were performed in the morning with the fasting patient in the supine position

Bromsulphalein (*Sulfobromophthalein* Hynson Westcott & Dunning) was given as a continuous infusion to 27 patients during a period of three hours Eleven of these cases received an infusion of an identical amount 2—3 days later this time preceded by a priming dose given in the course of 20—30 seconds The remaining 16 patients received a further *infusion with a different amount* 2—3 days later (one of the cases had three infusions with different amounts)

Bromsulphalein was determined in arterial blood samples taken at 10-minute intervals The experimental details have been reported elsewhere (19) So as to determine whether the concentration was constant or continuously rising the late part of the blood *concentration curve* was graphed and the slope was determined by regression The concentration was then assumed to be constant if the regression line was not significantly different from zero (t test 5 *per cent limit*)

The plasma clearance of bromsulphalein was calculated during constant concentration as the amount infused per minute divided by the average plasma concentration When the concentration was rising linearly the amount of bromsulphalein removed *from plasma* was estimated as the amount infused

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lein curve

Regression coeff (mg/100 ml/min x 10)	Mean error of regress on coeff	Average arterial concentration (mg/100 ml)	S D *	Plasma clearance (ml/min)	Amount removed (mg/min)
22	84	1 54	0 087	541	
19	63	1 37	0 078	586	
[illegible]	86	2 57	0 046	330	
81	170	2 36	0 090	344	
82	53	1 98	0 053	331	
96	6	2 18	0 067	267	
122	336	2 20	0 080	267	
325	287	3 08	0 043	197	
29	89	0 79	0 081	1 082	
13	56	1 19	0 042	704	
412	79	1 12	0 082		4 89
−44	12	1 04	0 013		5 03
85	42	0 72	0 032	761	
97	164	0 66	0 127	795	
2 300	154	4 94	0 118		6 58
2 508	141	6 69	0 110		6 50
1 726	235	3 32	0 237		7 63
[illegible]301	70	3 97	0 073		8 03
[illegible] 271	307	10 44	0 198		7 85
1 766	89	8 88	0 057		9 35
964	106	3 23	0 079		8 54
605	108	3 87	0 087		8 46

(p 0 05 t test)

periments is the long time before a constant concentration (e g Exp No 429—430) or a constant rise in concentration (e g Exp No 432—433) is reached This time seems to increase with the amount infused (a positive correlation is found between amount infused and this time determined graphically (r = 0 4 p < 0 01)) The average time for obtaining a steady state was about 120 minutes and varied from 30 to 210 minutes It is furthermore seen from the figures that the concentration of bromsulphalein fluctuated considerably the deviations around the regression line being greater than expected from the analytical error (*compare* tables I and II) In some cases (e g Exp No 442, 743 and 810) the variations appeared to be cyclic each cycle lasting for about 30

TABLE I The effect of a priming dose on bromsulphalein infusion curves

Exp No	Body weight (kg)	Amount infused (mg/min)	Priming dose (mg)	Regression of the bromsulph Interval (min from start)	No of samples
534	78 0	8 34		90–200	18
535	78 0	8 01	164	90–200	18
538	57 2	8 48		120–180	7
539	57 2	8 11	329	120–180	7
581	73 2	6 54		120–240	16
582	73 2	5 82	162	140–240	15
584	120 6	5 85		210–240	7
585	120 6	6 08	155	210–240	7
734	64 0	8 58		100–180	9
735	64 0	8 41	370	100–180	9
832	76 5	5 05		80–180	11
833	76 5	5 01	392	80–180	11
838	83 2	4 99		100–180	9
843	83 2	5 01	430	100–180	9
852	74 0	7 43		100–180	9
854	74 0	[1]7 43	382	100 180	9
863	77 0	8 30		80–180	10
864	77 0	8 15	414	80–180	10
865	85 3	10 10		110–180	8
867	85 3	[1]10 10	409	110–180	8
874	64 5	9 90		100–180	9
879	64 5	9 68	329	100–180	9

[1] Not analyzed [2] S D of experimental points around the regression line [3] Different from zero

minus that retained in the plasma, calculated as the rise in concentration per minute multiplied by the plasma volume, assumed to be 5 per cent of the body weight

The urine was voided after the experiment, and in the cases where no priming dose was given the average renal clearance of bromsulphalein was estimated from the average arterial concentration, assuming that the amount excreted per minute in the urine was constant during the experiment

Results

The experiments demonstrating the influence of an initial single injection on the elimination of bromsulphalein during infusion are shown in table I and in fig 1 The experiments in which different amounts was infused are seen in fig 2 and the data in table II

A general feature of most of these ex

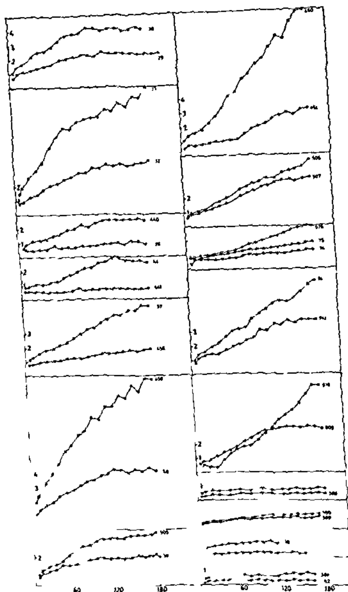


Fig 2 Infusion of bromsulphalein in different amounts Coordinates as fig 1

minutes The e changes often made the delimination of the curves difficult (e g Exp No 531)

The experiments with and without a priming dose show that the slope of the curve in each varies much It is however seen from table III that this variation may be accounted for by the day to-day changes found in similar experiments where no priming dose was given (19)

Fig 1 Infusion of bromsulphalein with (●) and without (0) a priming dose Abscissa time in minutes Ordinate concentration of bromsulphalein (mg/100 ml)

Mean error of regression coeff	Average arterial concentr (mg/100 ml)	S D *	Plasma clearance (ml/min)	Amount removed (mg/min)	Urinary excretion: Clearance (ml/min)	Urinary excretion: Amount eliminated (mg/min)
194	2 32	0 072	314			
110	4 04	0 084	298			
73	0 92	0 030	464		[illegible]	
59	2 63	0 025	347		5 5	
50	0 71	0 053	749		3 1	
151	2 72	0 104	323		2 8	
22	0 49	0 027	566		[illegible] 8	
38	0 8	0 012	361		4 9	
252	0 79	0 045	530		3 5	
103	1 00	0 052	517		3 4	
43	1 03	0 067	33			
5	0 31	0 036	410			
329	0 81	0 104	4 0			
22	0 31	0 034	579			
397	3 47	0 306	241	(8 13)	3 7	
2 0	7 9	0 245		13 40		0 03
4	4 20	0 126	275	(11 54)		
492	9 73	0 319		22 40		
143	2 12	0 057	331	7 01	2 9	
1 8	3 53	0 068		7 76		0 01
248	3 22	0 116	314	(10 17		
03	4 80	0 143		9 81		
46	1 67	0 030		7 25		0 08
143	4 40	0 093		8 73		0 20
[illegible]	8 24	0 245		9 59		0 09
492	2 63	0 132		6 84		0 04
	3 9	0 137		8 60		0 13
[illegible]	3 13	0 109		7 32		0 12
	1 16	0 036		4 88		0 04
	1 6	0 040		6 0		0 05
813	2 59	0 0 6		6 81		0 10
191	4 61	0 123		7 31		
[illegible]	5 49	0 134		8 95		

* S D of experimental points around the regression line

TABLE II Infusion of bromsulphalein in different doses

Exp No	Body weight (kg)	Amount infused (mg/min)	Regression of the bromsulphalein curve: Interval (min from start)	No of samples	Regression coeff (mg/100 ml/min x 10⁴)
429	56 4	7 26	110–210	11	50
430		12 02	100–190	9	–65
439	105 5	4 27	130–180	6	–87
440		8 79	130–180	6	84
441	60 5	5 29	80–180	11	–3
442		8 77	110–180	8	17
586	70 8	2 75	60–180	11	19
587		4 16	140–180	5	85
589	80 6	4 20	105–170	7	24
590		5 16	120–180	7	135
930	73 1	3 63	80–110	4	–49
933		1 27	80–150	7	–8
981	52 8	3 64	120–160	5	67
982		1 80	30–160	14	4
432	58.1	8 34	110–190	9	729
433		14 06	100–190	10	[1]2 283
458	60 0	11 54	110–180	8	12
459		23 32	110–180	8	[1]3 075
504	73 0	7 04	140–180	9	91
505		7 98	110–175	8	[1]615
809	85 9	10 12	120–180	7	–117
810		11 73	110–180	8	[1]4 481
456	77 5	7 45	110–180	8	[1]516
457		10 41	110–180	8	[1]1 760
460	73 1	11 58	110–180	8	[1]5 434
461		7 38	110–180	8	[1]1 509
506	96 0	9 46	110–180	8	[1]1 791
507		7 66	110–180	8	[1]708
574	61 7	4 98	110–180	8	[1]317
575		6 22	110–180	8	[1]493
576		7 36	110–180	7	[1]1 768
741	53 3	7 54	110–180	8	[1]805
743		9 71	110–180	8	[1]2 843

[1] Different from zero ($p < 0.05$, t test)

50 % (e g Exp No 809—810)

The urinary elimination of bromsulphalein, expressed as renal clearance in per cent of plasma clearance (cases with constant concentration) or as amount excreted in per cent of the amount removed from plasma (cases with rising concentration) was 0 9 % (s d 0 5) in the experiments with the lower amount infused In the experiments with a larger dose the urinary elimination rose significantly ($p < 0\ 01$) to 1 3 per cent

Discussion

1 *Bromsulphalein clearance*

During constant infusion the dye removing function of the liver can be expressed by a plasma clearance or an amount removed from plasma per time unit These two expressions are measures of the parameters of the removal mechanisms during different conditions the latter determines the maximal amount which can be removed and thus is a measure of the amount of functioning enzyme present while the first determines the intensity at which the removal mechanisms work at submaximal load

If the plasma clearance of bromsulphalein is to be of any value it must be independent of the concentration attained in the peripheral blood i e independent of the amount infused This has been examined by different methods in animals and man In measurements on rabbits Lewis (9) found proportionality between concentration and amount infused Pratt et al (13) found that a change in the amount infused giving concentrations from 1 to 4 mg/100 ml did not alter the hepatic extraction percentage in dogs and Combes et al (7) demonstrated that in dogs the hepatic uptake of bromsulphalein was roughly proportional to the peripheral concentration during the initial part of the infusions In man, Castenfors et al (6) found by hepatic venous catheterization that the extraction percentage was constant at concentrations below 2 mg/100 ml but diminished at higher concentrations This indicates that in man the clearance decreases with rising concentration above a certain limit Similar conclusions are reported by Brauer and Pessotti (3) from experiments in dogs They found that with infusions below 0 16 mg/kg/minute the clearance was constant and with larger infusions it was decreasing

The present experiments confirm that in man bromsulphalein clearance is not independent of the peripheral concentration but decreases with increasing arterial concentration In these studies the spontaneous variation in clearance (the causes of which have been discussed previously (19)) has been taken into consideration and a priming dose has been omitted The initial single injection has been claimed to saturate the extrahepatic removal of bromsulphalein (2) and to accelerate the time of equilibration The renal excretion, which is the only extrahepatic elimination that can be measured in man, was not saturated during the experiments (table II) In the dog Brauer et al (4) found no evidence for saturation during prolonged infusions In the present experiments a priming dose did not diminish the elimination significantly and the time of equilibration was not appreciably altered by the priming dose The usefulness of a priming dose thus cannot be confirmed by these experiments

Several causes of the decrease in

TABLE III Summary of the effect of amount infused on bromsulphalein clearance and on the amount removed from plasma during infusion

Arterial concentration	Type of experiment	No. of exp.	Ratio of 1st and 2nd experiments: Amount infused	Clearance or amount removed	(s.d.)
Constant	a) Same amount repeated	6	1.00	1.16	(0.40)
	b) as a) ± priming dose	6	1.03	1.15	(0.23)
	a) + b)	12	1.02	1.16	(0.35)
	c) Two different amounts	7	0.57	1.40	(0.45)
Rising	a) Same amount repeated	9	1.00	1.01	(0.04)
	b) as a) ± priming dose	4	1.01	0.95	(0.10)
	a) + b)	13	1.00	0.99	(0.05)
	c) Two different amounts	6	0.77	0.82	(0.06)

The variation of the clearance was about 35 % and that of the amount removed from plasma was about 6 %. In 6 subjects a plateau was obtained twice and in 4 subjects both curves were rising. In the remaining patient (Exp. No. 832—833) a steady state was probably not achieved during the experimental period. The time required for obtaining a constant or a constantly rising concentration does not seem to be altered by the priming dose.

In the experiments where different amounts were infused a constant concentration was reached twice in 7 of 16 subjects. It is seen from table III that an increase in the dose of about 75 % resulted in an average decrease in the clearance of about 70 %. When this alteration in clearance is compared with the day-to-day variation the difference is on the borderline of significance ($0.10 > p > 0.05$). In five subjects the concentration was steadily rising in both experiments. When the difference in the slopes of the concentration curves is expressed as amount removed from plasma per minute, it is found that with an increase in dose of 30 % the removal rate increased by 20 %. This is significant ($p < 0.01$) when compared to the day-to-day variation. The increase in amount removed with increasing amount infused is clearly seen in Exp. No. 574, 575, 576 (table II).

In the present series of infusions without a priming dose a constant concentration was reached in about half of the cases. The maximal dose allowable to avoid a rise in arterial concentration cannot easily be determined from these experiments. Cases with a constant concentration were encountered with doses of 12 mg/minute while a continuous rise in concentration occurred with infusion of 5 mg/minute. In four experiments a constant concentration was obtained with the lower dose while a continuous rise was seen when the dose was augmented at the average by

plasma concentration at equilibrium varies with the amount infused in a way similar to that experimentally found i e for a given increase in amount infused the concentration rises more It will be seen that this concentration is determined by all the constants of the model, and thus it is only possible to determine the individual constants in man by repeating the experiments with at least seven different doses This has not been feasible because of the small interval during which it is possible to obtain a constant concentration the spontaneous variations in the concentration taken into consideration, and because these prolonged infusions often cause a phlebitis which prohibits repetition of the experiment When a very low amount is infused the model gives a solution in which the concentration is proportional to the amount infused i e a true clearance can be determined However even this clearance does not measure a separate process of removal but contains all constants of the kinetic equations

B The maximal elimination of bromsulphalein

When different amounts are infused it is sometimes found that the lower dose results in a horizontal curve while the larger one gives a constant rise in the concentration (e g Exp Nos 741—743 809—810) This usually signifies that a step in the eliminating system is working at maximal speed whereby the removal from plasma becomes constant It has been thought that the limiting process was the uptake of bromsulphalein from plasma into the liver (Lm) Then this process could be measured as has been done in animals by Mason et al (10) and Taleisnik (14), and in man by Verschure (15) It is however seen from the present cases with different amounts infused that this maximal amount increases with the dose given which shows that a real limit for elimination is not reached The same has recently been found by Andersson et al (1) This might be caused by the use of an erroneous volume of distribution but if this volume is determined from two experiments with different doses incredibly high values are found even in relation to the combined intra and extravascular albumin space The changes cannot be explained by augmented urinary elimination at higher plasma concentrations because this is quantitatively unimportant

At present most work on the maximal removal of bromsulphalein is done according to the concept of Ingelfinger (8) who found that it was possible to get a constantly rising concentration with much smaller doses than earlier reported He interpreted this limit of elimination as a maximal transfer rate from liver to bile (Tm") As it was known that an increase in hepatic uptake could be demonstrated during a rising arterial concentration e g by a single injection, Wheeler et al (16) proposed a method for calculation of Tm by assuming that the amount eliminated from the blood in excess of Tm was proportional to the concentration Their experiments with different doses and comparison of the calculated Tm with that determined from the excretion in the bile confirmed the principle of the calculation However, the presence of metabolites of bromsulphalein in the bile makes the interpretation difficult and the experimental reports concerning the

plasma clearance with increasing load may be considered. That it is caused by saturation of the extrahepatic removal is — as just mentioned — unlikely. It might be due to the way in which the experiments were performed. As bromsulphalein may be stored in the liver for a considerable time and as the stored dye probably inhibits the further uptake from the blood, the clearance determined in a second experiment may be diminished by that performed first. The small (and insignificant) decrease in the experiments with a priming dose (table I) could be caused by this effect. Some of the experiments with different doses have been made by giving the greater amount in the first experiment, without however resulting in a smaller clearance when a smaller amount was given later on. It seems most likely that the dependence of clearance on the concentration is caused by the properties of the removal mechanisms. Changes in the hepatic blood flow from day to day cannot be excluded, but in other experiments with identical doses no correlation between the hepatic flow and clearance was found (19).

The long time necessary for reaching a constant arterial concentration is not compatible with the concept of a removal from the intravascular space by a simple mechanism. Wheeler et al. (17) stated that in dogs this time — determined as the time when the hepatic uptake of bromsulphalein became constant — was about 30 minutes, while Brauer and Pessotti (3) found longer times such as have now been found. It might be caused by a slow extravascular diffusion and a slow mixing with the extravascular plasma pool. Another explanation is possible. There is evidence that bromsulphalein once taken up in the liver may recirculate to plasma, and this might considerably augment the time required to reach a steady state. An indication of this phenomenon is the wave-like changes in concentration sometimes found before the concentration becomes constant, and the irregularity of the level of concentration, which causes the variation of the concentration to be about three times greater than would be expected from the analytical error. Other possible causes of this instability are fluctuations in the hepatic blood flow or extraction of bromsulphalein in the liver, changes in the flow of the vein used for the infusion, and variations in the amount infused (s.d. of the pump delivery was 1.4 per cent).

From the above-mentioned findings it seems possible that the kinetics of bromsulphalein removal during constant infusion are complex. Therefore, a model which uses enzyme-kinetic expressions and involves a recirculation of bromsulphalein from the liver back to plasma has been considered. The model has previously been applied to single injection experiments (20). No general solution of this model is possible, but in the *Appendix* it is shown that during certain restrictive conditions of the constants involved this model describes some of the experiments findings. It is found that the concentration in the liver during equilibrium depends only on the ratio between the volume of distributions in the liver and in plasma, and on the removal constants of the biliary excretion. It has not yet been possible to determine the volumes and the concentration in the liver for assessment of the last mentioned process. If the amount infused is kept sufficiently low the

Appendix

The mathematical description of the model of elimination in the case of continuous infusion

If the model for the elimination of bromsulphalein earlier presented is considered in relation to continuous infusion (20) the differential equations describing the system are the following

$$\text{1a)}\quad \frac{dc}{dt} = -\frac{LM_1\,c}{KM_1+c}+\frac{V_1}{V}\,f\,c_1+\frac{i}{V}$$

$$\text{1b)}\quad \frac{dc_1}{dt} = \frac{LM_1\,c}{KM_1+c}-f\,c_1+\frac{LM\,c}{KM_1+c}\,\frac{V}{V_1}$$

where

c is the concentration of the first compartment (plasma) with volume V

c_1 is the concentration in the second compartment (liver) with volume V_1

KM_1 and LM_1 are the Michaelis constant and the maximal velocity for the enzymatic elimination from the liver to the bile

f is the diffusion constant for the recirculation from the liver to the plasma

i is the amount infused per time unit.

The general solution of the equations (1) is not known to the authors but the nature of the solution depends strongly on the infusion dose i. Depending on whether i is less or greater than a certain value the system approaches a steady state or a state where c rises without limit. If in fact

$$2\quad i\ \ \frac{V}{2}\left\{\frac{V_1}{V}\ LM_2+\frac{V_1}{V}\ f\ KM_2+LM_1\right\}$$
$$-\frac{V}{2}\sqrt{\left(\frac{V_1}{V}\ LM_1+LM_1+\frac{V_1}{V}\ f\ KM_2\right)^2-4\ \frac{V_1}{V}\ LM_1\ LM_2}$$

the system approaches a steady state with the asymptotic values for concentration

$$\text{3a}\quad c\ \ \frac{KM_2\ i}{V\ LM_2-}$$

$$\text{3b}\quad c\ \ \frac{KM\ \ i(V_1\ LM_2-i+V_1\ f\ KM_2)}{(LM\ \ V-)(V_1\ LM_2-i)-V_1\ f\ LM_2\ i}$$

The first of these equations shows that at equilibrium the concentration c_1 depends only on the amount infused and on the constants for the liver-to-bile elimination. The second equation shows that the measurable concentration c depends in a complicated way on all the constants so that in general it is impossible to get information about the constants from.

During special limiting conditions i e $i \ll V\ LM_2$ and $\ll V\ LM_1$ equation 3b) takes the simpler form

$$\text{3c}\quad \frac{i\ A}{B\quad C}$$

where A, B and C are constants containing KM, LM_1, KM, KM_2 and f). This shows that the equilibrium concentration grows faster than in proportion to the amount infused when this is increased.

If further $\quad i \ll \frac{LM_1\ \ LM_2}{f\ \ KM_2}$

equation (3b) yields

$$\text{(3d)}\quad c = i\ \frac{A}{B}$$

Only under this condition does the concentration at equilibrium become proportional to the amount infused.

For amounts infused which are above the equilibrium condition of (2) it is found that the concentration c rises without limit.

(4) $c \to \infty$ for $t \to \infty$

whereas c_1 has the limit

$$5\quad \frac{1}{f}\left\{\frac{V}{V_1}\ LM-LM_2-f\ KM_2\right.$$
$$\left.\sqrt{\left(\frac{V}{V_1}\ LM_2\ \ LM_2-f\ KM_2\right)^2+4\ f\ \frac{i}{V_1}\ LM_1\ KM_2}\right\}$$

existence of a 'Tm", common too all metabolites, are conflicting (11, 12)

From the present experiments with rising arterial concentration, no conclusions regarding the evaluation of "Tm" can be drawn Some curves indicate that a steady state may not be attained within three hours of infusion (e g Exp No 459), and is rarely attained within the interval commonly used (one hour) in experiments with (Nos 539, 576, 585, 867) and without (Nos 430, 433, 505) a priming dose This will cause systematic errors in the determination of Tm", and the problem how to ascertain that the conditions necessary for the calculation are fulfilled, needs further study

The solution of the model in *Appendix* shows that when the concentration in plasma is linearly rising, the concentration in the liver becomes constant and independent of a further rise in the peripheral concentration Then the volume of the liver does not enter the calculation of the maximal removal, and the plasma volume should be calculable from the 'Tm" determined in two experiments with different amounts infused As this is not the case, the model is not correct or the conditions of a steady state are not reached It is seen from fig 3 that the constant concentration in the liver is not reached until some time after the slope of the peripheral curve becomes constant, but it is of course not known whether this has any biological significance

It should be stressed that the present model must only be regarded as one possibility and that alterations in its structure may improve it This relates especially to the process of recirculation which largely determines the solution, but more experimental details of this process and of the other biochemical processes which govern the elimination of bromsulphalein are necessary The experiments of Brauer (5), showing that during an infusion of bromsulphalein a sudden single injection is preferentially excreted in the bile, call for more elaborate models

Summary

Bromsulphalein elimination curves have been studied during continuous infusions lasting for three hours

The effect of a priming dose has been examined, and it is found that it does not influence the time of equilibrium nor the general shape of the curves

When infusions with different doses are given to normal individuals, it is found that the bromsulphalein clearance is not constant, but decreases with increasing amounts infused A model of the elimination of bromsulphalein during infusion is presented, incorporating as an essential feature a recirculation of bromsulphalein already eliminated No general solution of the model is possible, but during special circumstances the model gives a usable description of many of the experimental findings

When infusions are given in doses which make the peripheral concentration rise continuously, it is found that the time necessary for obtaining a linear rise often is considerable This makes the calculation of the maximal removal rate for bromsulphalein difficult, and this problem is discussed in relation to different methods for calculation of this value (Lm" and Tm")

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A Population Study of 50-year-old Men

An Analysis of the Non participation Group

By

Gosta Tibblin

In order to be able to draw safe conclusions from population studies the population must be representative and the selection must be made at random The group examined is not representative unless there is a participation of almost a hundred per cent

In Sweden such a representative population is easily obtained by the aid of the registers of the Revenue Office It is also easy to make a random selection The great difficulty as regards medical population studies is to get a high number of participants Experience has shown that it is often impossible to obtain complete participation Therefore one has to be content with 90 per cent or less This means in a study of disease prevalence all the non participants may suffer from one of the diseases which is the object of study To be able to interpret various statistical results in a more certain way it is desirable to analyse the group that did not participate and the possible circumstances connected with non participation An analysis of the non participation group becomes an important part of every population study

The size and composition of the non participation group are dependent both on the special features of the population examined and on the make up of the examination offered Analyses of the non participation group in one population study therefore cannot be applied to other studies Analyses of the non-participation group in population studies are scanty in literature A detailed study of the non participation group has been made by Lindegård et al (1) in connection with a medicopsychological questionnaire survey (1961)

As regards general health examination it has been suggested that the non participation group might be particularly ill and therefore most in need of a health examination In such cases an analysis of the non participation group will give the sources of error of the investigation,

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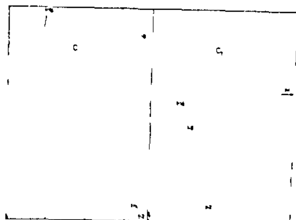


Fig. 3. Solution by electronic computer of a model of the elimination during infusion. c: plasma concentration. c_1: liver concentration. i: amount infused per time unit. LIM: limit for c_1 with $i = 8$ and 16. Abscissa: time (arbitrary units). Ordinate: concentration (arbitrary units).

The slope of the curve $c - c_{(i)}$ approaches the values

$$(6) \quad \frac{dc}{dt} \to \frac{i}{I} - LM_1 + f \cdot c_1 \cdot \frac{I_1}{I}$$

as follows from equation (1a). Since c_1 is independent of i, (6) shows that the slope of the asymptote for $c_{(i)}$ increases linearly with increasing i. Only if

$$LM_1 \geq \frac{I_1}{I} \cdot f \cdot c_1$$

is determination of LM_1 possible directly from the slope of $c_{(i)}$.

In fig. 3 an example is given of the course of the concentrations c and c_1 during variations in the amount infused (i). The curves are constructed from figures obtained on an electronic computer, using the following arbitrary constants for equations (1): $I = 3{,}500$, $I_1 = 700$, $LM_1 = 0.1$, $KM_1 = 1$, $LM_2 = 0.01$, $KM = 1$, $f = 0.1$.

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TABLE III Comparisons concerning income in Sw Kr between the men in the total material, the group examined and the various non participation groups (the Gothenburg Study 1963)

Income (Sw Kr)	< 12 000		12 000—14 000		15 000—18 000		> 19 000		No information	
	No	%	No	%	No	%	No	%	No	%
Total material (n=973)	267	27	240	25	196	20	225	23	45	5
Hospital group (n 855)	09	24	219	26	186	22	213	25	28	3
Non participation group (n 118)	58	49	21	18	10	9	12	10	17	14
Home visit group (n 40)	18	45	10	25	6	15	4	10	2	5
Non participation A (n 20)	5	25	3	15	4	20	3	15	5	25
Non participation B (n 58)	35	60	8	14	0	0	5	9	10	17

cipation A Those who for other reasons did not take part in the examination were put in a second group non participation B The latter group included 18 men who had announced their refusal to take part in the examination In general these men gave no specific motive for their position In no case was illness mentioned as the reason Over the telephone 6 persons promised to come but did not Thirty two non participants could not be contacted in any way and in 17 cases the address was incorrect and they could not be located

Results

Sex and age

The examined group and the non participation group were due to the make up of the investigation of the same sex and age

Income

The information about the income in 1961 of the men selected was acquired from the Revenue Office The total series was divided into four classes The classification was made in such a way that about one fourth fell into each class

There were considerable differences in income between on the one hand the total series and the hospital group and on the other the three non participation groups In the group visited at home about 70 % were found to be in the two lower income groups (< 15 000 Sw Kr/year) In non participation B (other reasons) 74 % of the men belonged to the two lower income groups There were also considerable differences as to those for whom no information (in most cases the same as no income) could be obtained from the registers In the non participation group there were about three times as many for whom no information could be obtained

Civil status

The information concerning civil status was obtained from the registers of the Revenue Office 80 % of the total number of men were married In the hospital group the number was 85 % The number in non participation B (other reasons) was 35 per cent (table IV)

TABLE IV Number of married men in the total material the hospital group and the various non participation groups (the Gothenburg Study 1963)

	No	%
Total material (n=973)	779	80
Hospital group (n=855)	27	85
Non participation group n 118	52	44
Home visit group n=40)	21	53
Non participation A (n 20)	11	55
Non participation B (n=58)	20	35

TABLE I Number of men examined at the Sahlgren's hospital, at home, and non examined (the Gothenburg Study, 1963)

	No	%
Hospital group	855	88
Home visit group	40	4
Non examined	78	8
Total	973	100

TABLE II Reasons for non participation of groups A and B (the Gothenburg Study, 1963)

	No
A Natural reasons	
Deceased	7
Admitted to hospital	4
Moved to another town	5
Away during 1963	4
B Other reasons	
Announced refusal to participate	18
Not kept promise to come	6
Not at home when visited	15
Wrong address	17
Other reasons	2
Total	78

as well as a description of those persons who do not accept the offer of a health examination

For natural reasons it is difficult to examine a group of people who refuse to participate Possible ways are to use information obtained from record offices or to record the attempts made to get in contact with these persons A third possibility is to visit them in their homes and in this way get an opinion of the characteristics of the non participants

A combined health examination and population study was carried out at Sahlgren's hospital in Gothenburg during 1963 The investigation is described in detail elsewhere (2) This report includes different aspects of the non participation group in that investigation The variables that could be studied concerning this group were sex, age, civil status, income, sobriety, place of birth, and morbidity A third of the non participants were visited at home and asked to give the motive for non participation

Material and methods

From the registers of the Revenue Office 973 men, all born in 1913 and living in Gothenburg, were selected at random 855 of these men were examined at Sahlgren's hospital during 1963 In the following they are called the hospital group (table I) During the autumn of the same year home visits were made to 40 men who had refused to take part in a hospital examination but agreed to be interviewed in their homes Several attempts were made to get in contact with the group of 78 persons who were neither examined at the hospital nor in their homes At least two attempts were made to reach them at home Their latest known addresses were collected from the Revenue Office and from the General Sickness Insurance Company In some cases attempts were made to contact them at their jobs

As regards this latter group it has partly been possible to compile the reasons for non participation and for the rest of the men in the non participation group the stage was registered at which the attempt to contact them broke down (table II) Those who for natural reasons were unable to take part in the examination i e those who were deceased those who had moved or been away in 1963 and those who for a longer period in the same year had been in hospitals or convalescent homes were put in a special group non parti

Gothenburg are for the total material and for the hospital group 46 and 47 % respectively In the non participating group 40 % were born outside Gothenburg The difference is not significant

Morbidity

All employees and self employed in Sweden above 16 years of age receive a daily sick allowance when they report a sickness lasting more than three days From the information of the General Sickness Insurance Office in Gothenburg the morbidity could be studied *Two groups of the total series were studied* A random sample drawn from the total material consisted of 95 men This group was compared with the total non participation group (118 men) The following arbitrary variables were studied number of men obtaining sick allowance once a year or less *number* of men obtaining sick allowance two or more times a year and number of men sick more than 30 days at one time a year Each year from 1955 to 1962 was accounted for separately Those who were granted invalidity pensions were assumed to have been ill continuously since the date of their retirement From table VII it is clear that the morbidity as expressed in number of men ill per year and in number of men with two periods or more of illness is not significantly different for the two groups As regards the long sickleaves (more than 30 days) on the other hand, the difference between the two groups is significant for the years of 1959 1960 and 1962

Pension

Twenty three men in the total material were registered as receiving invalidity pension In the non participation group 8 were pensioned χ^2 analysis shows this difference to be significant ($\chi^2 = 5.9$ $p < 0.025$) Yates correction has been used in the statistical studies The reason for retirement was mental disease in 13 cases and somatic disease in 10 cases All but one of those with somatic illness were examined at Sahlgren s hospital

The reason for non participation of the home visit group

In connection with the visits to their homes the non participants were questioned as to their reasons for not coming They were asked to give several reasons and to mention which one they considered the most important In

in the non participation group (n 118) (the Gothenburg Study 1963)

Non particip		Sample		Non particip		Sample		Non particip	
No		No	%	No	%	No	%	No	%
		1957				1958			
49	42	42	44	58	49	42	44	52	44
20	17	16	17	18	15	14	15	22	19
22	19	13	14	27	23	18	19	27	23
		1961				1962			
52	44	32	34	46	39	34	36	55	47
17	14	9	10	12	10	13	14	20	17
8	*24	12	13	27	23	10	11	27	*23

$\chi^2 = 4.249$ p 0 05

TABLE V Comparisons between the examined group and the various non participation groups as regards registration in the records of the Temperance Board in Gothenburg (the Gothenburg Study, 1963)

	No registered	%
Total material (n = 973)	232	24
Hospital group (n = 855)	179	21
Non participation group (n = 118)	53	45
Home-visit group (n = 40)	15	38
Non participation A (n = 20)	7	35
Non participation B (n = 58)	31	54

TABLE VI Place of birth of the men in the total material the hospital group and the various non participation groups the Gothenburg Study 1963)

	No born in Gothenburg	%
Total material (n = 973)	444	46
Hospital group (n = 855)	400	47
Non participation group (n = 118)	44	37
Home-visit group (n = 40)	16	40
Non participation A (n = 20)	7	35
Non participation B (n = 58)	21	36

Temperance

Data referring to excessive alcohol intake were obtained from the registers of the temperance board in Gothenburg There all offenders since 1920 are registered, even if the offence had been primarily registered elsewhere in Sweden To have been reported to the temperance board was used as a rough measurement of presence or absence of alcoholic problems among the men selected

24 % of the total number of men had records with this board The various non participation groups showed a considerably higher frequency and it was as high as 54 % for non participation B (other reasons) (table V)

Place of birth

To give a rough opinion on the mobility of the various groups their place of birth is recorded in table VI The numbers for those born in

TABLE VII Morbidity as seen in a sample drawn at random from the total material (n = 95) and

	Sample		Non particip		Sample	
	No	%	No	%	No	%
Sick allowance	1955				1956	
> One time	28	30	44	37	26	27
> Two times	8	8	12	10	14	15
> 30 days	10	11	23	20	12	13
Sick allowance	1959				1960	
> One time	30	32	46	39	29	31
> Two times	6	6	12	10	9	10
> 30 days	10	11	25	[1]21	12	13

[1] $\chi^2 = 4.355$, p 0.05

[2] $\chi^2 = 5.597$, p 0.05

burg excluded on the whole the possibility that they had been admitted to hospitals One might assume that the majority of them were at sea, in prison, in inebriates homes, or vagrants In 13 cases (11 %) the reason for non participation was illness or death For those cases hospital records or death-certificates have been available

It should be mentioned that Gothenburg is a harbour and the largest port of Sweden, which necessarily influences the composition of the population and may explain some of the results

The composition of the hospital group is well in keeping with the total material as regards variables examined In spite of this the non participation group differs significantly from the total material in several respects The explanation of this is that the non participation group is relatively small and therefore of less importance As to physical illnesses it is possible that phases of chronic alcoholism such as hepatic cirrhosis might be more frequent among the non participants The prevalence data concerning this disease might thus be too low for the examined group As to other physical illnesses there is reason to believe that the examined group gives a true picture of the material selected

In the discussion about the value of health examinations the idea has been expressed that the non participants might be especially in need of medical care because they might be considerably sicker than the examined group The results from this study give no support to that idea but indicate that they might be in need rather of socio-economic support.

Summary

In a combined health examination and population study of 973 50 year-old men the non participation group of 118 men has been analyzed One of the main reasons for non participation was a negative attitude towards medical care in general (38 %) Only a small number of the men were unable to come because of physical illness or death (11 %) The difference between the selected material and the group examined at the hospital is insignificant due to the relatively small size of the non participation group

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TABLE VIII Physical and mental illness among the pensioners in the hospital group and the various non participation groups (the Gothenburg Study, 1963)

Diagnosis	Hospital group	Non participation group	Total
Physical illness	9	1	10
Mental illness	6	7	13
Total	15	8	23

TABLE IX Motives for non participation of the men in the home visit group (the Gothenburg Study, 1963)

Negative attitude towards hospitals	12
Negative attitude towards doctors	8
Negative attitude towards the examination	8
Lack of time	9
Illness	2
Lack of interest	1
Total	40

table IX the main reasons for non participation are given A negative attitude towards hospitals, doctors, and the examination as such is predominant Twenty eight of the men questioned had this attitude Nine men said they did not have time, and 2 men had not been able to come due to illness

Discussion

In a combined health examination population study of 50-year-old men, living in Gothenburg, the non participation group differed from the total material regarding several of the variables examined The economic situation was different Most of the non participants had an income of less than 15,000 Sw Kr annually or lacked income Only 44 % were married They were to a larger extent found in the register of Temperance Board In the non participation group the pensioners were also more frequent In some important respects, however, they are equal to the total material Age and sex are the same due to the principles of the selection These two variables especially vary when the participating group is compared with the non participating group Their morbidity rate as measured by information supplied by the General Sickness Insurance Office is only higher as regards men on sick leave during a long period (30 days or more) The reason for this is certainly the great number of pensioners in the non participation group (8 men) as compared with the random sample (1 man)

One of the weaknesses of population studies is the lack of complete investigation of the whole group The reasons for non participation are therefore of interest In the present study a negative attitude towards doctors, hospitals, and health examinations seemed to be the main reason If the men who had refused to be examined (without giving any specific motive) are included in this latter group it will account for 37 % of the non participants Those who could not be reached amounted to 27 % A study of the hospital records in Gothen

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Hypertension in Two Sisters Caused by So-called Fibromuscular Hyperplasia of the Renal Arteries

By

JACOB HANSEN, CARL HOLTEN and J. V. THORBORG

Renal artery stenosis has during recent years been acknowledged as an important cause of arterial hypertension. The bilateral or unilateral narrowing of the renal arteries may be due to various pathological changes. Among these the so-called fibromuscular hyperplasia is of special interest for several reasons. We do not find this term adequate and shall return to this problem later in this paper. It differs fundamentally from the atherosclerotic changes which in some form or other are the most frequent causes of renal artery stenosis. The aetiology and pathogenesis must be different and pathologically it presents quite another picture. Atherosclerotic narrowing is most often found in the proximal third of the arteries, while fibromuscular hyperplasia is more apt to affect the distal parts. Contrary to atherosclerosis fibromuscular hyperplasia occurs mainly in young or middle-aged women and seems to be rare in males.

It was first described in 1938 by Leadbetter and Burkland (5) and the interest for this condition has of course greatly increased during recent years because of the rising interest of diagnosis and treatment of renal artery-stenosis which has been particularly aroused by the work of the Cleveland group (Poutasse, McCormack, Page, Dustan and others).

It is at present not possible to assess the frequency of fibromuscular hyperplasia as all publications represent a selection of cases. McCormack (Cleveland) has histologically examined 51 pathological arteries surgically removed. In seven cases segmental fibromuscular hyperplasia was found. Perloff et al. (9) performed renal arteriography in 110 hypertensive patients in 54 of whom vascular abnormalities were found. Among these 19 were unilateral and of these 5 had fibromuscular hyperplasia. Thirty-one were bilateral; among these there were 10 cases of fibromuscular hyperplasia. Hence out of about 50 cases of hypertension with vascular changes altogether 15 were found to be

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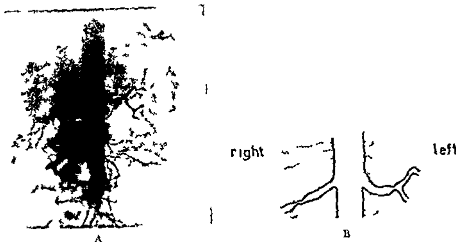


Fig 1 Case 1 A Aortography B Drawing from aortography

Physical exam nation

Weigl t 51 kg height 166 cm

Hea t exam nation sho ved regular action no murmurs

By *abdom nal auscul at on* a bruit synchron ous w th the pulse was heard to the left in the umb i cal reg on

Oph halmo copy sl owed fundus hyperton cus I

ECG sl gl t left preponderance

Renal funct on te ts were made several times Endogenous creat n ne clearance showed values of 76 to 97 ml/min The blood urea and serum creatinine were w thin normal l m ts Urinary output was normal Spec f c gra y was between 1 003 and 1 020 There was n erm ttent pro e nur a Attempt to measure different al renal funct on was un successful

Renal angiog aphy revealed def n te peripher al s enosis of both renal arteries most p onounced on tl e right f g 1 Dr C J Hodsc n Un vers ty College Hosp tal Lon don has seen tl e X ray f lms and sta ed his n pression that a narrow ng of other abdom nal visceral arter es e g the *splen c* artery was also seen

U nary excret on of *cate holamines* was normal Tl e urinary levels of 17 ketosteroids and 17 ketogen c s ero ds were normal Plasmahydrocortisone was within normal lim ts A skin and muscle b opsy from the left thigh showed no patholog cal changes of the blood vessels

On December 19 1961 the patient sudden ly collapsed and was adm tted to the Medical Department Central Hosp tal Randers in a moribund state She d ed on the following day from subarachno d haemorrhage

Case 2

The pat ent was born in 1927 She was well until 1950 when moderately elevated blood pressure and transient sl ght proteinuria were d scovered in association w th a *preg* nancy but her blood pressure was normal after parturit on n April 1951 On February 20 1953 she was admitted to the F rst Med cal Univers ty Department Århus for arte rial hypertens on During the period 1953 to 1962 she was re admitted 11 times and between the admiss ons she was controlled in the out pat ent department The patient s symptoms during these years were dominated by emot onal instab l ty w h unrest anxiety palp tat ons poor sleep and tinnitus There was sl gl t transient pyuria and bacteriuria on a few occasions and she experienced a tendency to fa nting for some time in 1959 These episodes occurred at intervals of 1—2 months On December 9 1959 she had a sudden attack w th loss of consciousness

due to fibromuscular hyperplasia These figures cannot give any impression of the incidence of fibromuscular hyperplasia as a cause of hypertension because the number of patients who had renal arteriography performed will be dependent on the number of cases with suspected renal hypertension But Perloff et al 's figures give some impression of the incidence of the condition among hypertensive cases in whom vascular changes could be demonstrated

The aetiology of fibromuscular hyperplasia is not known Several suggestions have been made and among these congenital abnormality has been considered particularly probable, so it may be hereditary Until now, however, no support of the belief that a familial disposition may be of importance has been given But the occurrence of fibromuscular hyperplasia in two sisters reported here may serve to shed some light on problems connected with this cause of hypertension and may suggest that exploration of familial and hereditary relatives in such cases might prove fruitful

Case reports

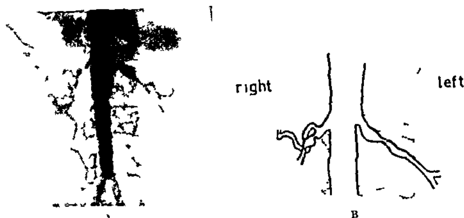
Case 1

The patient was born in 1929 At the ages of 5 and 16 she had scarlet fever, but otherwise no previous disease has been reported During the period 1949—1950 she was admitted five times to the Jutland Maternity Hospital in Århus, for metropathia hæmorrhagica Four pregnancies all terminated in spontaneous abortion The last pregnancy was in 1959, the three months premature infant lived for six days In October 1960 she was treated at home for a slight urinary tract infection

Present illness

Hypertension was first diagnosed in 1949 at the Jutland Maternity Hospital During her admissions in 1949—50, she presented a rather labile blood pressure varying between 180 to 140 systolic and 90 to 80 diastolic During admission for spontaneous abortion in 1959, her blood pressure was recorded in the range 220—165 systolic and 105—100 diastolic The patient was admitted five times during 1961 to the Medical Department, Central Hospital, Randers, for arterial hypertension Three days before her first admission in January 1961, she complained of increasing left sided headache, nausea and vomiting She had an episode of hypertensive encephalopathy on the day of admission manifested by loss of consciousness, generalized clonic convulsions and hyperventilation Her blood pressure was 240/120 and treatment with reserpine and mecamylamine was initiated In April 1961, the diagnosis of bilateral renal artery stenosis was made by means of aortography She was transferred to the Surgical Department T, University Hospital Århus Operation, however, was abstained from because of the presence of bilateral stenosis The patient was transferred to the First Medical University Department Århus, for further examination The dominating symptoms were nervousness, anxiety and attacks of palpitations

She was treated with guanethidine and hydrochlorothiazide with added potassium chloride She reacted violently to guanethidine treatment which she thought produced physical and mental fatigue and after discharge she stopped taking the drug on her own initiative in October 1961 Blood pressure before treatment was 230—210/130—115, and during treatment in sitting position was 140/80 and recumbent 180/100 Antihypertensive therapy was only intermittently maintained between hospitalizations The patient again and again discontinued the medication, because of the previously mentioned side-effects



F g 2 Case 2 A Aortography B Draw ng from stereo-aortography

onstr ct on was local zed 1 5 cm from the aorta It was a l ttle longer but less sharply def ned than that of the right s de No stenosis was seen n the other contrastf lled ar eries fig 2)

Other exam nations

Several exam nations of the urine for cate holamines revealed normal values The ur nary excretion of 17 keto and 17 keto-ge uc s eroids as normal

Cou e

Except for treatment w th methyldopa heraj y had been disappointing probably because the j at ent only sporad cally took t e prescr bed med cine when not n hosp tal Fo this eason among others operat on was re j la ed and discussed with the pat ent wl o n ally refused but af er the sudden t a l f l sis er changed l er m nd On the lem e of aor ographs the right renal artery was l el e ed to be n ore narrowed than the l f and tl e perfo mance of a plast c opera n tl is s de seemed to be w th n reacl She was ope ated upon on Noven ber 8 1962 *u mary of the po t of the operat on* The a a as found to be w thout atheromatous han es Tl e r gl t renal artery was stenot c aj j ox ma ely 5 m lateral to t e aorta and t e l f renal artery was s eno c from ts s n The left renal artery di ded just distally to the stenot c area into two branches of about equal size There was a *pressure* grad ent of approximately 200 mm Hg across the stenotic area in the right renal artery and *approximately 150 mm Hg* across that of the left. The right kidney was approximately 8 cm long and the left approximately 12 cm The surfaces of both kidneys were smooth A bifurcated teflon graft was anastomosed end to-side to the aorta a few centimetres distal to the origin of the renal arteries The two branches were crossed over the renal veins and anastomosed end to-side with the renal arteries on the right artery 5—6 cm from the origin and on the left artery a few mm from the origin The kidneys were cooled with ice during clamp ng

Comment on the operat ve find ngs

The greater pressure grad ent across the right renal artery conf rmed the results of the Howard Rapoport test since tl e test suggest ed more pronounced ischaemia of the right k dney The d fference in size between the right and left kidneys was found to be greater at operat on than est mated on intravenous urograpl y

Po t-ope ative cour e

Renal insuff ciency developed post-operative ly The h ghest blood urea value was 226 mg 100 ml and was measured on the f fth

strong tonic contractions of the peripheral muscles lasting a few minutes and followed by profound fatigue There was no involuntary voiding of urine or stools The attack resembled an episode of an acute hypertensive encephalopathy and blood pressure measured immediately after the attack was 215/115 The diagnosis of renal artery stenosis — possibly bilateral — was suggested by an aortogram taken in September 1961, and confirmed in November 1962 by stereoaortography

An almost constantly elevated, but somewhat labile blood pressure was found from February 1953 onwards The maximum pressure recorded before treatment was 240/140 Antihypertensive therapy was begun in December 1954, and a variety of drugs was given including reserpine hydralazine and mecamylamine Results were never satisfactory and therapy was discontinued in August 1961 at the time of admission to hospital to elucidate whether renal artery stenosis could be demonstrated The impulse to the study was the fact that renal artery stenosis had been found in her sister However, anti hypertensive therapy was again started on September 2 1962 this time with methyldopa 250 mg t i d The systolic blood pressure fell under this treatment from 240—210 to 180—150 mm Hg but the diastolic pressure was unaffected and remained around 100 mm Hg Methyldopa therapy was discontinued on November 3, 1962

Physical examination Weight 47 kg height 166 cm

Heart No sign of enlargement A soft systolic murmur was heard over the entire praecordium

The abdominal aorta could be felt pulsating and a high frequency grade III—IV bruit could be heard over it especially a little above and to the left of the umbilicus.

Ophthalmoscopy showed fundus hypertonicus II

ECG Within normal limits

Pre-operative renal function tests Occasional, slight proteinuria, specific gravity up to 1 024

Creatinine clearance Between 72 and 98 ml/min Serum creatinine 0 9 to 1 2 mg/100 ml

Blood urea 11 to 21 mg/100 ml Serum electrolytes normal

Differential renal function test was performed according to Hayward and Rapoport

This test rests on the observation that the sodium concentration is lower and the creatinine concentration higher in the urine from an ischaemic kidney than in the urine from a non ischaemic kidney Urine is collected from each ureter and the ratio calculated by the following equation

$$\frac{\text{sodium concentration in left ureter urine}}{\text{creatinine concentation in left ureter urine}} \cdot \frac{\text{creatinine concentration in right ureter urine}}{\text{sodium concentration in right urether urine}}$$

Urine was collected during three 1/2 hour periods

1st period $\frac{79}{11} \cdot \frac{85}{39}$ 15 8 2nd $\frac{75}{9} \cdot \frac{80}{61} =$ 10 5 3rd $\frac{54}{6\ 5} \cdot \frac{28}{24}$ 9 7

The normal range for this index is between 0 62 and 1 62 The index will thus be high if ischaemia of the right kidney is present and low if ischaemia of the left is found The values obtained in this patient demonstrated that the right kidney was ischaemic as compared with the left

X ray studies

Chest films Normal findings

Aortography (September 8 1961) demonstrated a stenosis of the left and probably of the right renal artery

Intravenous urography (March 28 1962) The right kidney was a little smaller than the left otherwise the findings were within normal limits

Stereoaortography (October 11 1962) Filling of the renal coeliac mesenteric and iliac arteries was seen The entire study lasted 10 seconds Short areas of constriction of both renal arteries were clearly seen in all the films On the right side the constriction started 1 cm from the aorta and extended just distal to the origin of the supra renal artery It seemed to be about 1 mm long On the left side the

coel ac a tery \ as markedly stenotic measuring only 1 ɔ mm n d ameter whereas the open ng of the super or mesenteric artery as normally cal brated Tl e openings of the o her arter es orig nat ng from the aorta er normal *The splenic a e y* was cut open and found to be macroscop cally normal *The enal arter es* were dissected free and found to ha e smooth contours and est mated to be of normal s ze no thicken ngs or aneurisms were seen (fig 3) Tl e arteries of the neck of the pel is and the femoral arteries were opened and found normal

The k dneys were of normal shape but the rigl t was smaller than the left It we gl ed 109 g wh le the ve ght of the left was 167 g The surface of the right kidney was finely d ffusely granulated with a sl ghtly adherent capsule Tl e parencl ymal tissue was slightly reduced w th a normal proport on between t e cortex and the medulla The tissue was darker than normal due to a pronounced I yperaen a, especially marked n the pyra n ds The smaller vessels d d not seem to be pathologically sclerotic The surface of the left k dney was smooth with an easily st pp ng capsule The parenchymal tissue was of normal w dth being markedly w der t an on the right s de The smaller arteries were es mated to be somewhat sclerot c

The b a n At the base of the bra n extend ng somewhat up along tl e con ex ty of the t e n spheres and downwards along tl e brain s em o tl e foramen magnum a rather ex ens e recent haemorrhage was found The t asal essels were embedded n large masses of t l od but no rup tures or aneurisms could be f un l No haemorrhage was found n the t a n substance or n the en ricular s stem ex ept a sn all blood clot n tl e lumen of the fo r h entricle



F g 4 Case I Lateral part of the right renal artery F bro c h ckened int ma Internal elast c lamella irregular and partly fragmented Elast n sta n × 42



F g 5 Case I \ al e l ke hyperplasia of he media and n ma of artery branch n the renal h lum No e the th n almos qu e a roph c wall at one s de of the l yperplas a Haema oxyl n-eos n 42

Microscopically

Renal arteries Sect ons were taken med ally lose t he aorta at the mid level laterally at he ram f cat on near the l lum and from tl e re al hilum just before tl e entrance into tl e renal tissue In the medial and mid level sec ns only n nor patholog cal changes are found consisting of moderate f brous changes of the med a, where the muscle fibres to a certain degree are nterrupted and replaced by collageneous tissue and irregular del cate elast c f bres partly orig nat ng from the outer elast c la er which forms a th ck partly f brous mantle around the vessel. The h ckness of the wall is un form with no nt mal prol ferat on and no changes of the nternal elast c lamella In the lateral sec t ons, at the level of the ram f cation the changes are more consp cious. The branches of the arteries are at some parts of normal appearance but at places both the intima and med a are rregularly thickened The intima is here formed by a rather dense f brous tissue partly mixed with the irregularly re dupl cated and spl it up internal elastic la-



Fig 3 Case 1 The retroperitoneal organs from behind Note the smaller size of the right kidney, the smooth outer contours of the renal arteries and the slight atheromatosis of the aorta

post operative day Thereafter the blood urea fell to 61 mg/100 ml

The post operative azotaemia was probably the result of renal ischaemia secondary to clamping of the renal arteries at operation, however absorption of blood from a retroperitoneal haematoma probably played a part Except for slightly lower levels on the first post-operative day the blood pressure after operation was little changed as compared with that before surgery and averaged around 200/120 mm Hg

On November 20 1962 the patient was transferred to the First Medical University Department After surgery she was very tired and emotionally instable She became suddenly worse at 6 00 a m on November 25 1962 She was found to be unconscious with large non reactive pupils and absent deep tendon reflexes The spinal fluid was haemorrhagic The patient died 18 hours later The immediate cause of death was subarachnoid haemorrhage

Epicrises

The case histories of these two sisters were remarkably alike, they had slightly or moderately elevated blood pressures in connection with pregnancy in case 2 and a gynaecological disorder in case 1 Intermittent hypertension in both was found at an early age, case 2 at 25 years, case 1 at 21 years Both at an early stage had fainting and convulsions attacks due to hypertensive encephalopathy Both were mentally unstable and in neither patient could treatment with antihypertensive drugs be successfully carried out because of side effects which they could not tolerate In both patients X-ray examination showed small differences in kidney size, and aortography demonstrated renal artery stenosis in both cases, apparently more marked in the right renal arteries

The hypertension in neither of them could be characterized as very severe as their eye grounds revealed only a low grade retinopathy Electrocardiography only intermittently in case 1 showed slight left preponderance, in case 2 the ECGs were virtually normal Renal function was not impaired in either case

The younger sister died from a subarachnoid haemorrhage at the age of 32 The elder sister was operated on at the age of 35 and also had subarachnoid haemorrhage which was the immediate cause of her death

Autopsy (abstract) case 1

The heart was slightly enlarged weighing 360 g (body weight 55 kg) due to a moderate hypertrophy of the left ventricle The cut section of the myocardium showed no abnormalities *The coronary arteries* were patent with only slight traces of atheromatosis No ectasia or narrowing of *the aorta* was found In the thoracic part the intima was questionably diffusely thickened, and in the abdominal part slight atheromatous patches and streaks were seen The ostium of *the*

coeliac artery was markedly stenotic measuring only 1 5 mm in diameter, whereas the opening of the superior mesenteric artery was normally calibrated The openings of the other arteries originating from the aorta were normal *The splenic artery* was cut open and found to be macroscopically normal *The renal arteries* were dissected free and found to have smooth contours and estimated to be of normal size no thickenings or aneurisms were seen (fig 3) The arteries of the neck of the pelvis and the femoral arteries were opened and found normal

The kidneys were of normal shape but the right was smaller than the left It weighed 109 g while the weight of the left was 167 g The surface of the right kidney was finely diffusely granulated with a slightly adherent capsule The parenchymal tissue was slightly reduced with a normal proportion between the cortex and the medulla. The tissue was darker than normal due to a pronounced hyperaemia especially marked in the pyramids The smaller vessels did not seem to be pathologically sclerotic The surface of the left kidney was smooth with an easily stripping capsule The parenchymal tissue was of normal width being markedly wider than on the right side The smaller arteries were estimated to be somewhat sclerotic

The brain At the base of the brain extending somewhat up along the convexity of the hemispheres and downwards along the brain stem to the foramen magnum a rather extensive recent haemorrhage was found The basal vessels were embedded in large masses of blood but no ruptures or aneurisms could be found No haemorrhage was found in the brain substance or in the ventricular system except a small blood clot in the lumen of the fourth ventricle

Microscopically

Renal arteries Sections were taken medially close to the aorta at the mid level laterally at the ramification near the hilum and from the renal hilum just before the entrance into the renal tissue In the medial and mid level sections only minor pathological changes are found consisting of moderate fibrotic changes



Fig 4 Case 1 Lateral part of the right renal artery Fibrotic thickened intima Internal elastic lamella irregular and partly fragmented Elastin stain × 42



Fig 5 Case 1 Valve like hyperplasia of the media and intima of artery branch in the renal hilum Note the thin almost quite atrophic wall at one side of the hyperplasia Haematoxylin eosin × 42

of the media where the muscle fibres to a certain degree are interrupted and replaced by collageneous tissue and irregular delicate elastic fibres partly originating from the outer elastic layer which forms a thick partly fibrous mantle around the vessel The thickness of the wall is uniform with no intimal proliferation and no changes of the internal elastic lamella In the lateral sections at the level of the ramification the changes are more conspicious The branches of the arteries are at some parts of normal appearance but at places both the intima and media are irregularly thickened The intima is here formed by a rather dense fibrous tissue partly mixed with the irregularly reduplicated and split up internal elastic la



Fig 6 Case I The coeliac artery The lumen is irregularly narrowed due to thick fibrous intimal cushions and hypertrophy of the media which at other sites is thin and atrophic Adventitia greatly thickened Elastin stain × 23



Fig 7 Case I The superior mesenteric artery The lumen is highly irregular and partly narrowed due to varying hyperplasia and thinning of the media Elastin stain × 23

mella (fig 4) The hyperplastic media is fibrotic and has degenerated in the same way as seen in the medial sections The adventitial coat is formed by a thick layer of collagenous tissue mixed with elastic fibres In one of the branches close to the renal parenchyma in the hilum of the right kidney a remarkable focal narrowing is found At this place the media is markedly hyperplastic with an overlying cushion like thickening of the intima At one side of the narrowing the wall of the artery is quite thin and fibrotic with a small outpouching of the lumen, although no real aneurism is seen (fig 5) *The coeliac artery* The sections have been taken at a level close to the aorta and show very conspicuous changes in all layers (fig 6) The lumen is unevenly narrowed and widened by extensive fibrous thickenings of the intima and changing hypertrophic and atrophic parts of the media, in which the muscle fibres are broken and partly replaced by fibrous tissue The internal elastic lamella is split up and is partly disintegrating The outer coat consists of a very thick multilayered elastic and collagenous tissue *The superior mesenteric artery* In one of the sections the lumen is highly irregular in outline, mostly due to a varying thickness of the media, the elastic tissue here being without major changes (fig 7) At other places, however, the external elastic layer has degenerated and is broken with rather extensive defects. *The splenic artery* is examined at two levels, the most lateral section being without changes The medial section is partly changed in the same way as described under the lateral sections of the renal arteries with fibrous intimal thickening and splitting of the internal elastic lamella *Aorta* A pronounced atheromatous and fibrous thickening of the intima is found in the sections from the abdominal aorta Moreover a moderate ingrowth of capillaries into the media accompanied by a slight infiltration of lymphocytes and plasma cells is present Sections from *the femoral artery*, *the internal carotid artery* and *middle cerebral artery* are unremarkable In many smaller arteries a simple diffuse hyperplastic sclerosis of the hypertensive type is present in the sections from the various organs

Right kidney Most glomeruli are without conspicuous changes however a slight diffuse thickening of the capillary tufts and the Bowmann s capsule is found In several glomeruli the juxtaglomerular cells and the macula densa are seen to be rather well developed (fig 8) This feature has not been statistically evaluated The capsular spaces and the convoluted tubules of the cortex are slightly widened containing a small amount of proteinaceous material A moderate or well marked diffuse hyperplastic sclerosis is found in the arcuate and interlobular arteries in one minor branch with an excen



Fig 8 Case 1 To the left a glomerulus with prominent juxtaglomerular body To the right a glomerulus with prominent macula densa Haematoxylin-eosin, × 300

tric hyperplasia and splitting of the elastic tissue *Left kidney* Histologically similar slight changes as those in the right kidney are found but in addition some of the afferent arterioles show a moderate sclerosis *Suprarenal gland* Except for a rather pronounced glomerular zone and a fairly marked arteriolosclerosis no changes *Skin from the abdominal wall* In the corium a moderate perivascular infiltration of lymphocytes and plasma cells together with a small number of eosinophils is present

Main autopsy diagnoses of case 1

So-called fibromuscular hyperplasia of the abdominal visceral arteries (coeliac artery renal arteries splenic artery and superior mesenteric artery) Slight ischaemic contraction of the right kidney General hypertensive arterial and arteriolar sclerosis Slight hypertrophy of the left ventricle of the heart Subarachnoid haemorrhage extensive (immediate cause of death)

Autopsy (abstract) case 2

The heart was slightly enlarged weighing 350 g (body weight 43 kg) due to a moderate hypertrophy of the left ventricle Otherwise normal findings *The coronary arteries* were patent with only traces of atheromatosis In *the aorta* only very slight atheromatosis was present mainly localized to the lumbar part The stem of the teflon graft was anastomosed to the anterior wall below the orifices of the renal arteries The two branches were connected with the renal arteries about 0 5 cm (left) and 2 5 cm (right) laterally to their orifices The lumen of the graft was patent

Renal arteries The orifices were of normal width but 12 mm laterally in the right artery

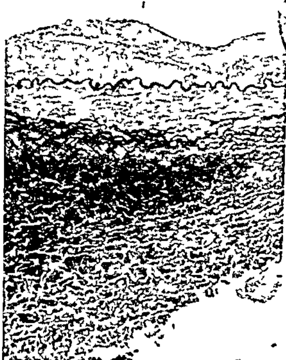


Fig 9 Case 2 Right renal artery Adventitia greatly thickened Pronounced intimal fibrosis Internal elastic lamella unbroken Elastin stain × 42

a 2—3 mm long stenosis was present, hardly allowing the passage of a 1 mm thick sound A similar stenosis was present in the left artery in close relationship to the anastomosis No pronounced atheromatosis was found

The kidneys were slightly diminished in size, the right weighing 105 g, the left 110 g both were of normal shape The surfaces were smooth although with small granulated areas The cut surfaces were unremarkable The pelves ureters and urinary bladder showed no changes

The brain No intracerebral haemorrhage was found but at the base a moderate, recent subarachnoid haemorrhage was present extending upwards along the Sylvian fissures No macroscopically discernible abnormality of the cerebral vessels especially no aneurisms

In *the peritoneum* a small number of blood clots were found, mainly deeply located along the root of the mesentery presumably originating from the retroperitoneal tissue at the level of the right kidney No pertinent findings in other organs

Microscopically

Renal arteries Due to secondary inflammatory and fibrotic changes from the operation only some sections from the right artery can be used for description The most impressive change is that the adventitial coat is greatly thickened, at some places 2—3 times the thickness of the other parts of the wall (fig 9) It consists of many layers of coarse elastic fibres from the external elastic layer, merging externally with connective tissue The media is not definitely hyperplastic, but the smooth muscle of this layer is diffusely mixed with and replaced by collagenous tissue and focally interspersed with coils of elastic fibres In several places the internal elastic lamella is fragmented The intima is diffusely thickened which may be part of the disease, but it cannot be ruled out that it may represent a recently organized thrombus *Superior mesenteric artery* The media is not hyperplastic but slightly and diffusely fibrotic The changes are only very moderate, the intima is normal or focally slightly thickened, and there are some degenerative changes of the external elastic lamella with splitting up and fragmentation of the fibrils *Carotid artery* One section is without changes but two other sections probably just above the bifurcation show a very marked asymmetrical atheromatosis of the intima The elastic tissue seems to be heavily degenerated and fragmented and mixed with collagenous tissue in varying proportions *Aorta* Slight atheromatous thickening of the intima No definite changes of the media except for small capillary proliferations into the outer layers accompanied by a small number of lymphocytes *Cerebral arteries* Several smaller and larger branches from the basal vessels show no pathological changes *Other vessels examined* The coeliac artery iliac artery, right coronary artery, a small artery from the dorsum pedis and in addition the vessels

in the sections from the various organs No specific changes are found, except a diffuse hyperplastic sclerosis of the smaller branches which should be considered secondary to the hypertension

The kidneys Post mortem autolysis is very pronounced so that finer details are lost The following minor changes are noted a slight arteriolosclerosis of some of the afferent arterioles and a slight diffuse thickening of Bowmann s capsule Small scattered interstitial lymphocytic infiltrations and a few hyalinized glomeruli

Sections from various other organs did not show important changes

Main autopsy diagnoses of case 2

Bilateral stenosis of the renal arteries (macroscopically) By pass operation done with transplantation of Teflon graft from aorta to both renal arteries So-called fibromuscular hyperplasia of the right renal artery and superior mesenteric artery (?) (microscopically) General hypertensive arterial and arteriolar sclerosis Slight hypertrophy of the left ventricle of the heart Subarachnoid haemorrhage extensive (immediate cause of death)

Additional biopsies from case 2

2 months prior to death a biopsy was made from a superficial branch of the temporal artery from muscle and from the skin

Definite medial hypertrophy was found in both the temporal artery and in the small arteries of the muscle consistent with the permanent hypertension Moreover in some sections from the temporal artery a small area of focal degeneration of the intima and internal elastic lamella was found superimposed by a small deposit of calcium

Biopsies were made from both kidneys at operation 18 days before death The tissue from the right kidney contained cortex including 8 glomeruli one of which was hyalinized the others being without definite changes No arteriolosclerosis was found but a small interlobular artery showed some slight diffuse medial hyperplasia The tubules were slightly dilated containing a small amount of granular material The tissue from the left kidney was insufficient

Comment on the pathological findings

The so-called fibromuscular hyperplasia, in most cases, has been described involving the renal arteries only, however, Poutasse et al (10) found changes in the coeliac and superior mesenteric artery also This assumed prevalence to the renal arteries may be real, but it may also largely be due to the fact, that the kidneys are far more sensitive to reduction of the blood supply than other viscera, so that a narrowing of the renal vessels will give rise to clinical symptoms before a stenosis of other arteries of the coeliac artery does so Moreover only few autopsy reports have been published

The morphological changes of the involved arteries may be found in all layers of the vessel the intima may be thickened by fibrous deposition the changes of the media, to which the condition owes its name consist of diffuse or focal hyperplasia but also reductions or even defects of the layer The elastic tissue, which in most cases is involved also, shows proliferative and degenerative phenomena with reduplications, coiling and focal or extensive loss of fibres The adventitia may be thickened so that a periarterial fibrous stenosis may develop The changes of the arteries seem to be of a definite segmental distribution and may include post stenotic berry like aneurisms (Palubinskas and Wylie (8)) Special attention to the degenerative changes of the elastic tissue as a patho

genetical factor has been paid by J D Hamilton in the paper by Yendt et al (16)

As emphasized by Wood and Borges (14) the natural history of the non-atherosclerotic stenosing renal artery disease has not been well defined, and it may be possible, that it represents more than a single entity

Concerning the two almost identical cases here described, the histopathological changes were best demonstrated in case 1 This does not necessarily mean, that the disease in this case was more advanced and more widely distributed As the lesions are of a definite segmental distribution in the arteries and as in none of the cases was a systematic serial-section examination done, some lesions may easily have been missed The findings especially in case 1 indicate however that the disease is not limited to the renal arteries, in fact, the most pronounced and characteristic changes were found in the proximal part of the coeliac artery Other arteries involved in case 1 were the superior mesenteric artery and the splenic artery Concerning case 2, the histopathological changes of the right renal artery in comparison with the stenoses indicate, that the patient suffered from the same disease, although the most typical finding is, that the stenosis is located in the lateral part of the artery The distribution of the lesions of the other arteries cannot be satisfactorily described The findings in the superior mesenteric artery suggest that this artery was also involved, but the changes consisted mostly of a moderate degeneration of the elastic tissue, and this may be seen in other conditions or even in normal mesenteric vessels The changes of the carotid artery was very marked, but as a pronounced atheromatosis was superimposed here, we cannot with certainty classify these changes

The valve like hyperplasia found in one of the large interlobar branches in the right renal hilum in case 1 deserves a special comment It has a strong resemblance to the intimal cushions, which have been described in the cerebral arteries as a normal finding even in young persons (Hassler (1)) Thus it may be a pathological exaggeration of an otherwise normal structure in the vessel wall, but in our case, however, the wall of the artery at one side of the hyperplasia was quite thin and fibrotic, suggesting the beginning of a post stenotic aneurism Defects of the media are well-known in the cerebral arteries, and Hassler (2) has also described them in other muscular arteries, especially in the splenic, renal, mesenteric and coronary arteries and even suggested that these defects may have significance in the development of some aneurisms of obscure aetiology

The origin of the subarachnoid haemorrhage was in neither case localized No distinct aneurism was found The explanation which we should like to put forward is that focal defects in the elastic tissue of the cerebral arteries have been present, and one of them has given way during a hypertensive crisis We find some support for this surmise in the fact, that focal degeneration of the internal elastic lamella had been demonstrated in the biopsy specimen from the temporal artery from case 2

The aetiology of this rather rare disease is not known, and our investigations do not permit of any conclusion The pathological features are quite different from ordinary atherosclerosis and have nothing in common with other known diseases of the vessels e g periarteritis nodosa or giant cell arteritis No inflammatory cells or necroses were found in connection with the lesions

From the above description it is clear that the term fibromuscular hyperplasia of the renal arteries is not adequate, as it has been demonstrated that all layers of the arterial wall may be involved, and that the lesions of the media are not limited to hyperplasia Furthermore the observations of Poutasse et al (10 11) and the observations here reported clearly show that the characteristic changes are not limited to the renal arteries but may also be present in other visceral arteries e g the coeliac superior mesenteric, and splenic arteries We should propose as a better term non atherosclerotic dysplasia of the visceral arteries

Our two cases occurred in two sisters Occurrence of the disease causing hypertension in siblings is just mentioned, but not discussed in a paper by Wood and Borges (14) (A 25 year old female patient had a 22 year-old brother with hypertension) We have not found such cases in any other publications

In both our cases hypertension was established at an early age and the blood pressure remained moderately elevated except for sporadic hypertensive crises in both The duration of known hypertension was 10—12 years and in both subarachnoid haemorrhage was the immediate cause of death In both bilateral renal artery stenosis was found and in both the histological examination revealed a picture which apparently was of identical nature, corresponding to what has been described in the literature as fibromuscular hyperplasia (or rather non atherosclerotic arterial dysplasia) The almost identical clinical course of the illness in connection with the pathological changes which doubtless are of the same nature should draw attention to the possibility that thorough analysis for familial occurrence of non atherosclerotic dysplasia causing hypertension might shed light on this comparatively rare cause of arterial hypertension

Summary

Hypertension in two sisters due to non-atherosclerotic arterial dysplasia (so-called fibromuscular hyperplasia) is described as to clinical course and pathological findings

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The Thiocyanate Suppression Test in Thyroid Disorders

By

Thorkild Friis and Svend Norregård

In 1936 Barker (1) reported that in some patients on thiocyanate (Rodanid) medication for hypertension he had observed development of goitre and signs of myxoedema In 1944 it was demonstrated by Franklin et al (2) that thiocyanate interferes with thyroid function by preventing the concentration of the iodide ion in the gland counteracting the propensity of the follicular cells to take up iodide

These observations remained little heeded by clinicians until Mitchell and O Rourke (5) in 1960 performed a detailed study of this mechanism Measuring the thyroidal uptake of radioactive iodine they found that serum levels exceeding 5 mg potassium thiocyanate per 100 ml abolished the uptake of iodine in 8 euthyroid patients but not in 3 hyperthyroid patients

In 1961 Mitchell O Rourke and Harden (6) reported that continued investigations on 25 euthyroid and 8 hyperthyroid patients had confirmed their first results Potassium thiocyanate had resulted in a 24 hour iodine uptake which averaged less than 10 % of the test does in euthyroid and more than 20 % in the hyperthyroid patients

There is no satisfactory explanation of these results on the basis of our present knowledge concerning thyroid function Possibly they are due merely to a turn over of thiocyanate so much more rapid in a hyperfunctioning gland that the effective intracellular concentration is reduced (Sanchez Martin and Mitchell 1960 (8))

Inspired by the investigations cited Sanchez Martin et al (9) tried to work out a thyroid function test according to the suppression principle Following administration of 3 g potassium thiocyanate by mouth in the course of 2 days they found a marked fall of the 24 hour iodine uptake to less than 20 % of the dose in 9 euthyroid patients and in 28 patients with diffuse or adenomatous, non toxic goitre In contrast no values

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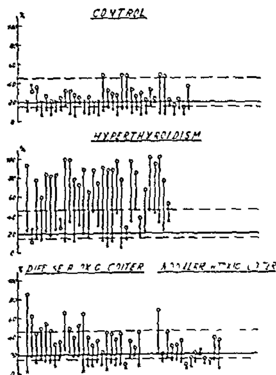


Fig 1 4-hour ^{131}I uptakes by the thyroid gland before and during administration of potassium thiocyanate. O Before administration of potassium thiocyanate \ During administration of potassium thiocyanate

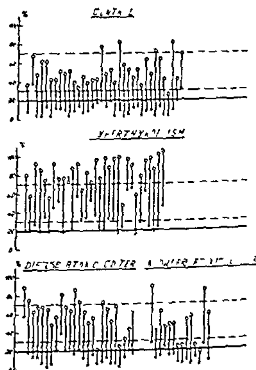


Fig 2 24-hour ^{131}I uptakes by the thyroid gland before and during administration of potassium thiocyanate O Before administration of potassium thiocyanate \ During administration of potassium thiocyanate

below 20 % were found among 17 patients with thyrotoxicosis

These authors observed only negligible side effects of thiocyanate in the form of dyspepsia in a few cases They believe that this test is preferable to the tri iodothyronine suppression test in being considerably quicker and better tolerated by thyrotoxic patients

We have now tested this method on a Danish series, comparing the results with those obtained by the tri iodothyronine suppression test, as reported by Friis (3) and by Østergård Kristensen and associates (11) Our main object was to evaluate whether the test is of diagnostic value in cases where the use of other diagnostic procedures has left doubt concerning the presence of thyrotoxicosis

Material

A total of 117 in patients were investigated Among this group 35 had no endocrine disease, in particular no thyroid disorder 31 had thyrotoxicosis according to the ordinary clinical and laboratory criteria 25 had diffuse non toxic goitre and 14 had nodular non toxic goitre while 3 were euthyroid following thyroidectomy for thyrotoxicosis Moreover 9 patients on thiouracil medication were tested

Method

Determination of the 4-hour and 24-hour thyroidal uptake of ^{131}I was carried out by the usual technique after oral administration of

TABLE I Mean value standard deviation and range of various parameters of thyroid function together with the result of the potassium thiocyanate suppression test

Group of patients	No of patients	4-hour uptake	24-hour uptake	PBI[131] (<0 3% per l serum)	Suppression to <20% uptake: 4 hour uptake No	Suppression to <20% uptake: 24 hour uptake No
Control patients	35	29 1±8 59 (14 6 –49 2)	50 9±13 72 (26 2–81 9)	0 15±0 22 (0–0 65)	33 (94 6%)	33 (94 6%)
Hyperthyroid	31	76 1±24 11 (10 7–107)	83 4±15 34 (48 0–105 0)	1 27±1 46 (0 01–5 43)	13 (41 9%)	6 (19 4%)
Diffuse non toxic goitre	25	43 4±15 90 (9 7–89 6)	60 5±17 03 (21 3–89 4)	0 13±0 17 (0–0 59)	23 (92 0%)	19 (76 0 %)
Nodular non toxic goitre	14	29 1±15 63 (8 4–68 4)	48 9±21 61 (18 9–90 0)	0 16±0 15 (0–0 48)	12 (85 7%)	11 (79 0%)

the isotope (Friis and Korsgård Christensen 1959 (4)) On the day after this test had been completed the subjects were given potassium thiocyanate 1/2 g four times by mouth and on the next day again 1 g at 8 a m At 9 a m they received another test dose of [131]I after the residual activity in the thyroid gland had been determined The 4 hour and 24 hour uptakes by the gland were again measured the residual activity and 87 % of the residual activity being subtracted from the 1st and 2nd measurements respectively as the radioactive iodine remaining in the gland was considered to have an effective half life of 6 days

Results

In the *35 control patients* the findings were a follows (figs 1 and 2 table I) for the 4 hour and 24 hour uptakes the mean values were similar to previous findings mean = 29 1 % and 50 9 % of the dose, (Friis and Korsgård Christensen 1959 (4) Pettersen et al 1964 (7) Strange 1959 (10)) 5 and 4 respectively were above the normal range which is 15—45 % of the dose for the 4 hour uptake and 30—70 % for the 24 hour uptake (4 7) while 1 + 1 were below For PB[131]I mean = 0 15 % of the dose per litre serum 4 were above the upper limit It may be added that 9 had a basal metabolic rate exceeding + 10 % and 5 of less than – 10 % The result of the suppression test showed that the 24 hour uptake decreased to below 20 % in 33 patients The same applied to the 4 hour uptake

As regards the *31 hyperthyroid* patients (figs 1 and 2, table I) the mean 4 hour and 24 hour uptake values were 76 1 and 83 4 % which is in keeping with previous findings in this country 4 and 5 values respectively were below the upper limit of normal For PB[131]I mean = 1 27 % of the dose per litre serum five were in the normal range In the suppression test 6 were found to be below

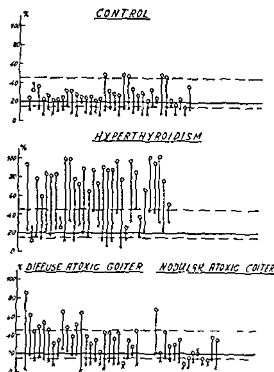


Fig 1 4 hour ^{131}I uptakes by the thyroid gland before and during administration of potassium thiocyanate O Before administration of potassium thiocyanate ╲ During administration of potassium thiocyanate

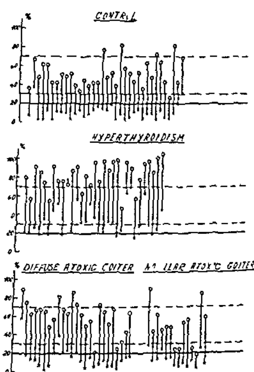


Fig 2 24 hour ^{131}I uptakes by the thyroid gland before and during administration of potassium thiocyanate O Before administration of potassium thiocyanate ╲ During administration of potassium thiocyanate

below 20 % were found among 17 patients with thyrotoxicosis

These authors observed only negligible side effects of thiocyanate, in the form of dyspepsia in a few cases They believe that this test is preferable to the tri iodothyronine suppression test in being considerably quicker and better tolerated by thyrotoxic patients

We have now tested this method on a Danish series, comparing the results with those obtained by the tri iodo thyronine suppression test, as reported by Friis (3) and by Ostergård Kristensen and associates (11) Our main object was to evaluate whether the test is of diagnostic value in cases where the use of other diagnostic procedures has left doubt concerning the presence of thyrotoxicosis

Material

A total of 117 in patients were investigated Among this group 35 had no endocrine disease, in particular no thyroid disorder 31 had thyrotoxicosis according to the ordinary clinical and laboratory criteria, 25 had diffuse non toxic goitre and 14 had nodular non toxic goitre while 3 were euthyroid following thyroidectomy for thyrotoxicosis Moreover 9 patients on thiouracil medication were tested

Method

Determination of the 4 hour and 24 hour thyroidal uptake of ^{131}I was carried out by the usual technique after oral administration of

TABLE II Number of patients in the various groups who "overlapped" during the potassium thiocyanate test

		Limit (%)		
		15	18	20
4-hour uptake				
Control group	(35)	6	3	2
Hyperthyroid	(31)	8	10	13
Diffuse non toxic goitre	(25)	9	5	2
Nodular non toxic goitre	(14)	3	2	2
Total		26	20	19
24 hour uptake				
Control group	(35)	5	4	2
Hyperthyroid	(31)	0	2	6
Diffuse non toxic goitre	(25)	11	6	6
Nodular non toxic goitre	(14)	3	3	3
Total		19	15	17

test (Ostergard Kristensen et al (11)) a single patient was also found to "overlap" in that one out of 13 control patients was in the thyrotoxic range While the 4 hour and 24 hour determinations were of similar usefulness in the T_3 test, the 4 hour measurements were considerably inferior to the 24 hour measurements in the potassium thiocyanate test

The question is now whether there is a more suitable limit than that selected by Sanchez Martin et al (9)) If it is set at 15 % of the administered dose there would be no hyperthyroid patients in the normal range with respect to the 24 hour measurements, but 5 of the control group would fall within the hyperthyroid range (table II) If the limit is set at 18 % 4 of the control patients would be in the hyperthyroid range while 2 of the hyperthyroid patients would be in the normal range In that event then, 6 out of 66 patients would overlap as compared with 5 and 8 when the 15 % and 20 % limits are taken Similar considerations concerning the 4 hour measurements show (table II) that, as already mentioned the 20 % limit puts 2 control patients into the hyperthyroid range and 13 hyperthyroid patients in the normal range If the limit is set at 18 % these numbers will be 3 and 10 patients and if the limit is set at 15 % they will be 6 and 8 patients, i e inferior to the result with the 24 hour uptakes Altogether then, the 20 % limit was somewhat inferior to the 15 % and 18 % limits for these two groups of patients

With regard to the non toxic goitres, the results of the potassium thiocyanate test are on the whole like those of the T_3 suppression test in Friis series (3)

20 % in the 24-hour uptake In the 4-hour uptake test 13 were below 20 %

Among the 25 *patients with diffuse, non toxic goitre* (figs 1 and 2, table I), the mean values were 43 4 %, 60 5 %, and 0 13 % for the 4-hour uptake, 24-hour uptake, and $PB^{131}I$ respectively, likewise in accordance with previous findings 9 and 6 had an uptake in excess of the upper limit of normal, 1 and 2 were below the normal range, and 4 had increased $PB^{131}I$ It is a well-known phenomenon that in this country non-toxic, diffuse goitres may show tendency to increased uptake In the suppression test the 24-hour uptake could be suppressed to less than 20 % in 19 patients With respect to the 4-hour uptake, 23 could be suppressed to less than 20 %

In the *14 patients with non toxic adenoma* the mean values of the 3 parameters were found to be 29 1 %, 48 9 %, and 0 16 % 1, 2, and 2 respectively were above the normal range, 2 and 4 below (4 hour and 24-hour uptake) In the suppression test 11 could be suppressed to less than 20 % with respect to 24 hour uptake For the 4-hour uptake the corresponding number was 12

The material includes, moreover, 3 *patients rendered euthyroid by thyroidectomy for hyperthyroidism* In these cases the findings were normal

Out of the 9 patients on methyl thiouracil therapy the 4-hour and 24 hour uptakes could be supressed to below the 20 % limit in 5 by potassium thiocyanate

Discussion

It seems reasonable to compare the value of the potassium thiocyanate suppression test with that of the tri iodothyronine suppression test In a study from 1963 Friis (3) found tri iodothyronine to suppress thyroid function in 26 of 27 control patients In that study a K value was used $= \frac{K_1 + K_2}{2}$ in which K_1 was

$$\frac{\text{4 hour uptake of } ^{131}\text{I before administration of } T_3}{\text{4-hour uptake of } ^{131}\text{I during administration of } T_3} \text{ and } K_2 \text{ was}$$

$$\frac{\text{24-hour uptake of } ^{131}\text{I before administration of } T_3}{\text{24-hour uptake of } ^{131}\text{I during administration of } T_3}$$

The limit between euthyroid and hyperthyroid was found to be 1 40, euthyroid subjects having values above and hyperthyroid subjects values below this level Thus, in the control patients the two tests were of equal value (5 4 % and 4 1 % respectively behaving as hyperthyroids in the potassium thiocyanate and T_3 suppression tests) As regards the hyperthyroid patients, on the other hand, the potassium thiocyanate test was somewhat inferior to the T_3 suppression test With the former, 6 out of 31 were in the normal range (< 20 % uptake) when using the 24 hour uptake (19 4 %) as a criterion With use of the 4 hour measurements 41 9 % were in the normal range With the T_3 suppression test one of the 34 hyperthyroid subjects could not be suppressed by tri iodothyronine (2 9 %) In another Danish study on the T_3 suppression

thyroid range With regard to the setting of the limit 18 % and 20 % must be considered to be those which give rise to least overlapping Sanchez Martin et al (9) reported 20 % and this also accords with our experience

However, the 15 % as well as the 20 % limits may be used for diagnostic purposes as follows

A 24 hour uptake of less than 15 % following administration of potassium thiocyanate definitely militates against hyperthyroidism and a 4 hour uptake exceeding 20 % after potassium thiocyanate indicates hyperthyroidism with a 90 % certainty

Summary

The ability of thiocyanate to depress the thyroidal uptake of radioactive iodine has been utilized by Sanchez Martin et al in a clinical suppression test analogous to the tri iodothyronine suppression test After administration of 3 g potassium thiocyanate they observed suppression of the 24 hour ^{131}I uptake to less than 20 % of the administered dose in euthyroid subjects but never in thyrotoxic patients The test is considerably quicker than the T_3 suppression test and does not give rise to side effects in thyrotoxic patients

This test has now been tried on a Danish material of 117 patients 35 of whom were control patients without endocrine diseases, 31 were hyperthyroid and 39 had non toxic goitre

The results do not quite accord with those of Sanchez Martin et al as there was rather a big overlap between euthyroid and hyperthyroid subjects, both with the 4 hour and with the 24 hour uptake The use of 15 % and 18 % limits for the radioactive iodine uptake instead of 20 % failed to give a better distinction between the two groups

Nevertheless, the test is of diagnostic value, when interpreted as follows a 24 hour uptake of less than 15 % following administration of potassium thiocyanate definitely militates against a diagnosis of hyperthyroidism, and a 4-hour uptake of more than 20 % following administration of potassium thiocyanate indicates hyperthyroidism with a 90 % certainty

No side effects were observed

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By potassium thiocyanate the I^{131} uptake by 19 patients (76.0 %) could be suppressed to below 20 % of the dose in the 24-hour uptake. By the T_3 test 84 % could be suppressed. The 4-hour uptake was more advantageous as far as the potassium thiocyanate test is concerned, as 23 could be suppressed (92 %). For the nodular goitres the corresponding values in the potassium thiocyanate test were 79 % and 86 % (at 24 and 4 hours) (table I), while the T_3 test was less advantageous, suppressing only 67 %. In this connection it must be mentioned that Ostergard Kristensen et al. (11) found that none of the 11 diffuse non-toxic goitres came within the hyperthyroid range in the T_3 suppression test. In other words, this is at variance with Friis' series (3). A comparison of the 20 % limit with the 18 % and 15 % limits shows that in this respect the 20 % limit is most advantageous (table II).

With regard to the mechanism in the potassium thiocyanate test, the explanation is possibly that, as already stated, potassium thiocyanate is more rapidly metabolized in patients with hyperthyroidism than in others, so that the serum level in hyperthyroid persons does not become high enough to suppress the uptake of ^{131}I as completely as in normal subjects. It might also be imagined simply that the uptake in hyperthyroid patients does not reach as low levels as in others, as it is already higher. The mean decrease in the 24-hour uptake was 41.0 % for the control group, 47.4 % for the hyperthyroid group, 45.6 % for the group with diffuse, non-toxic goitre, and 37.0 % for the nodular goitres. Mutually, these values do not differ significantly, although there was a tendency for the hyperthyroid values to decrease most. A contributory cause to the observation that the hyperthyroid subjects were frequently not below the 20 % limit may then be that *a priori* their uptake is higher, although of course other factors may be operative.

Conclusion

In accordance with previous observations we found potassium thiocyanate to exert a markedly suppressive action upon the ^{131}I uptake by the thyroid gland, but we could not fully confirm Sanchez-Martin et al.'s (9) experience of their potassium thiocyanate test in that we did not find this test as valuable as the T_3 suppression test for distinguishing between hyper- and eu-thyroid patients. While the potassium thiocyanate test showed an overlap of 8—12 % between the control and hyperthyroid group, depending upon whether the 15 % or the 20 % limit is used in the 24 hour measurements, this overlap was only about 3 % for the T_3 test. Furthermore, the 4 hour uptake was even poorer with the potassium thiocyanate test, in which the overlap was approx. 20—23 %. In non-toxic goitre the results were similar to those obtained with the T_3 test, 24 % and 21 % of the diffuse and nodular goitres respectively falling within the hyperthyroid range when the findings are based upon the 24 hour values. When the 4 hour values were used, the findings were somewhat more advantageous, as only 8 % and 14 % respectively fell within the hyper-

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Propranolol (Inderal) in the Management of Adams-Stokes Syndrome in Childhood

By

ALF WENNEVOLD ERIK SANDØE and JOHANNES C MELCHIOR

The activity of the sympathetic nervous system is passed on to muscle and gland cells via two different receptors alfa and beta receptors (1) The only receptors in the myocardium are beta receptors which are associated with excitatory function Thus blocking of the beta receptors will antagonise the myocardial response to catecholamines and decrease myocardial irritability

In recent years blocking of the adrenergic beta receptors has found increasing use in the treatment og cardiac arrhythmias The first drug used clinically with success was pronethalol (Alderlin Nethalide) (3) Due to side effects from the central nervous system in patients and to toxic effects in mice which developed lymphosarcomas and reticulum-cell sarcomas, the clinical use of this drug had to be restricted Recently another adrenergic beta receptor antagonist has been introduced propranolol (Inderal) (2)

Propranolol has the same pharmacological properties as pronethalol and is — weight by weight — about ten times more active In the effective blocking dose few side effects have been reported, and in animal experiments no carcinogenic effect was revealed (2 6)

In this paper it is sought to assess the value of propranolol in the control of Adams Stokes seizures due to tachyarrhythmias in children Two children with such seizures were treated The effect of the drug was followed by electrocardiograms during and after exercise during treatment the myocardial irritability decreased and the drug managed to control the attacks to some extent

Case reports

I PATIENT S A (Department of Medicine B record no 668 64-65) Girl born 1952 was admitted on September 16th, 1964 for treatment of Adams-Stokes syndrome

She was adopted 6 hours after birth and no information concerning her family or delivery was available The birth weight was 3 000 g and growth and development were normal Apart from the common childhood diseases

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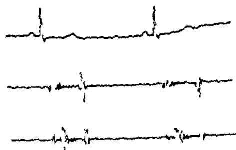


Fig 1 Phonocardiogram from the 2nd left intercostal space showing vibratory functional murmur

Finally the adrenergic beta receptor antagonist propranolol (Inderal) was tried with good result (table I). Although the arrhythmia during exercise was not completely suppressed, it took a longer time for it to develop and sinus rhythm ensued faster following the exercise.

The patient was discharged on a dose of 20 mg three times daily and returned for a short investigation 1 and 2 1/2 months later. She was during her whole admission given phenobarbital but this was discontinued at her discharge.

Follow-up. According to her mother's information during the first 2 months the patient was asymptomatic even if she did not reduce her physical activity. The electroencephalogram was unchanged one month after discharge.

At the first visit 1 month after discharge exercise on the bicycle ergometer with the usual load showed no effect of propranolol (table I) but it turned out that the patient had taken her first dose in the morning at 7 and her next dose at 3 p.m. a few minutes before the exercise test. The test was repeated 1 1/2 hours later and the usual good result was obtained (table I). It was therefore thought that the bloodlevel of propranolol was insufficient in the first test as actually 6 hours had elapsed since the morning dose.

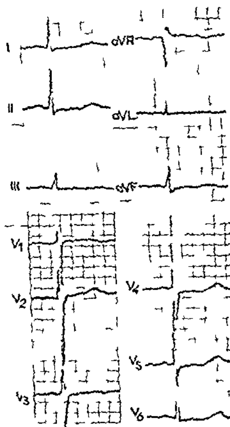


Fig 2 ECG at rest

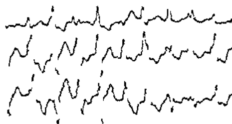


Fig 3 Leads V... V... and V... of ECG immediately after exercise on bicycle ergometer: runs of multifocal ventricular extrasystoles

hence her dosage was changed to 20 mg four times daily.

Four weeks later she became tired and drowsy and 2 1/2 months after discharge

she had been well until 1960 She then had a bad attack of measles with high fever and cerebral affection for a few days, and she had to stay in bed for 3 weeks Three months later she had her first attack of unconsciousness while playing, without convulsions, it lasted one minute, but she had to rest for one hour before she felt well again Within the next few months she had two similar attacks, and after the last attack her doctor, who was then called for the first time, noticed bradycardia with a pulse rate of 48

She was admitted to the regional hospital, where a sinus rhythm of 40—48 was found After exercise the electrocardiogram showed runs of multifocal ventricular extra systoles, the patient being clinically unaffected She was treated with atropine and ephedrine with slight increase in the heart rate, and was discharged During the next few months she had several spells of dizziness with pallor daily, usually in connection with exertion The heart rate gradually decreased and again reached the value of 40—48 which persisted

During 1961 and 1962 she had about one attack of unconsciousness per month but in 1963 her attacks increased in frequency and severity, now often resembling grand mal with convulsions The attacks were now also provoked by emotional disturbances

In June 1963 she was again admitted to the regional hospital for 4 months An electrocardiogram during the last part of an attack was interpreted as showing paroxysmal ventricular tachycardia and multifocal extra systoles Treatment with quinidine was started but had no effect, nor had reserpin The electroencephalogram which at first was normal, gradually changed to show some abnormality, which was thought to be due to the cerebral anoxia during the attacks She was discharged on atropine and phenobarbital

The remainder of the year 1963 and the first half of 1964 she remained well having only a few short lasting attacks However in the fall of 1964 she had a severe attack with convulsions and cyanosis lasting 40 minutes, her pulse could not be palpated She was then referred to the University Clinic of Paediatrics and later to the Department of Medicine B, Righospitalet, Copenhagen, for further treatment

Physical examination revealed a healthy looking, normally developed girl There were no signs of neurological disease At heart auscultation a grade 2 medium pitched systolic murmur of the "vibratory" type (fig 1) was heard to the left of the sternum with maximum intensity in the third left intercostal space The second heart sound was normal The heart rate was 48 The electrocardiogram at rest showed sinus bradycardia (fig 2) The chest roentgenogram was normal Right heart catheterization showed normal conditions The electroencephalogram was moderately abnormal (too low dominant activity with frequency of 6—7 hz)

An electrocardiogram obtained while the patient exercised on a bicycle ergometer showed within one minute after the beginning of the exercise runs of multifocal ventricular extra systoles (fig 3) which persisted till 4—6 minutes after the completion of the exercise, and which were followed by bigemeni for another 6—9 minutes whereafter sinus rhythm was restored (table I) No signs of cerebral ischemia were observed

Treatment The diagnosis of Adams Stokes attacks due to tachyarrhythmia was thus confirmed and during the next 2 months a trial of different anti arrhythmic drugs was performed while the effect was observed on the electrocardiogram during almost daily exercise on a bicycle ergometer (table I) The atropine which she had taken during several years was initially stopped, while a small dose of phenobarbital was continued throughout the trial mainly in order to restrict her physical activity somewhat

No effect was found of procainamide gilurytmal (Ajmalin) antazoline (Antistina) phenytoin and procainamide in combination with atropine Quinidine was not tried since it previously had increased the frequency of her attacks

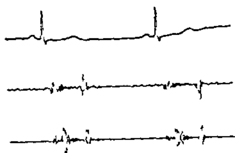


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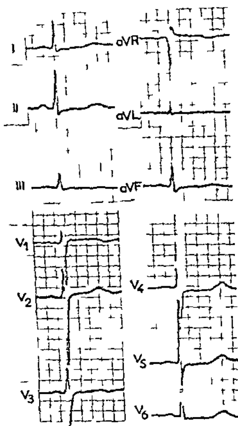


Fig 2 ECG at rest



Fig 3 Leads V V4 and V of ECG immediately after exercise on bicycle ergometer runs of multifocal ventricular extrasystoles

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Four weeks later she became tired and drowsy and 2 1/2 months after discharge

TABLE I Response to propranolol of arrhythmia during exercise. The work load at every test was 300 kgm/min for 1 min followed by 400 kgm/min for 2 min

1964 Date	Medicamentation	Onset of extra systoles after start of exercise: Few (min)	Bigeminy (min)	Runs (min)	Ending of runs and bigeminy after end of exercise (min)	Reappearance of sinus rhythm after end of exercise (min)
Sept 19–Oct 15	Range of 16 exercise tests during treatment with different drugs[1]			Immed to 1 min	4–6	10–13
Oct 20	1/2 hour after propranolol 10 mg			1/2	2	7
	1 1/2 hour after propranolol 10 mg		1	None		7
Oct 21	1/2 hour after propranolol 20 mg			1 1/2	4	11
	1 1/2 hour after propranolol 20 mg		2	None		5
Oct 22	1/2 hour after propranolol 20 mg	Immed		2	2	8
	1 1/2 hour after propranolol 20 mg		2 1/2	None		5
	4 hours after propranolol 20 mg		2	None		4
Oct 23	2 hours after propranolol 30 mg		1 1/2	None		3
	4 hours after propranolol 30 mg		1 1/2	None		6
Oct 24	No medication	1/2		1 1/2	1	13
Oct 27 1 00 p m	Propranolol 15 mg × 3 from Oct 26 Last dose at noon		1	None		7
Oct 29 1 00 p m	Propranolol 20 mg × 3 daily Last dose at 12 30 p m	1	2 1/2	None		[illegible]
Oct 31 4 tests	Propranolol 20 mg × 3 daily (6 15 a m noon 5 30 p m)					
	1 At 8 15 a m (2 hours after 1st dose)		1	None		6
	2 At 10 30 a m (4 hours after 1st dose)	1/2	1	2 1/2	Immed	6
	3 At 1 30 p m (1 1/2 hour after 2nd dose)		1/2	2 1/2	Immed	4
	4 At 4 45 p m (5 hours after 2nd dose)	1 1/2	1 1/2	None		7
Nov 30 2 tests	Propranolol 20 mg × 3 daily (7 a m , noon 5 30 p m)					
	1 At 1 20 p m (2nd dose that day at 1 15 p m)	Immed	1/2	1	3	14
	2 At 2 30 p m (1 1/2 hour after 2nd dose)		1	None		6

Table I Cont.

1 65 Da e	Med camenta on	Onset of ex ra systoles af er start of exerc se			I nd ng of runs and o s t of b g , m af er end of exerc se	Reappeara ce of a n s t ly ll af er end of exerc se
		Few m n	B g mem m	Runs m n	m n	m
Jan 18 12 4 p m	Propranolol 20 mg 4 da ly 7 a m 11 a m 2 p m 5 30 p m Las dose at 11 a m		I	None		7
F br 18 1 30 p m	Propranolol 15 mg × 4 da l 7 a m 10 a m 2 p m 5 0 p m) I ast dose at 10 a m	1	2	?	1 /	7
Feb 2? 1 p m	No propranolol s n e 20th February		Immed	1	6	14
Mar h 1 1 p m	S n e Febr 2 h p oprano ol 10 mg 4 da l 7 a m 10 a m 2 p m 5 30 p m Las dose at 10 a m		Immed	1	3	12
Ma h 3 3 e s	P op anolol 10 mg 4 da ly 7 a m 10 a m 2 p m 5 30 p m					
	1 At 11 a m 1 hour af e 2nd dose			1	4	14
	2 A 12 2 hours af er 2nd dose		*	1 /	3	9
	2 A 1 p m 3 hours af er 2nd dose			1 ,	2	6

Proca nam d gdurv nal Ajmal n an azol ne (An s na phenyto n and proca nam de n com b na on h a rop ne

he had a ew sho t last ng attack of uncon sc ousness She was seen again and an exerc se est howed he usual eff ct of propranolol Her pulse rate at rest was found to be 30 and as the drows ness had pers sted the dose as reduced to 15 mg four t mes daily The pulse ra e ncreased to 40—48 per minute but the dro s ness persisted and after another sl ght attack she was readmitted 3 1/2 n onths after discharge

The drug was stopped and w th n 24 hours her t redness disappeared A lower dose of propranolol was now tried 10 mg four times da ly but this had no or only slight ant arrhythm c effect as judged from the eletrocard og am during exercise

TABLE II Response to propranolol of arrhythmia during exercise The work load at the first exercise tests (Oct 20—Oct 23) was 200 kgm/min for 3 min, afterwards the load was stepped up to 250 kgm/min for 1 min followed by 300 kgm/min for another min and 350 kgm/min for another min

1964 Date	Medicamentation	Onset of extra systoles after start of exercise		Reappearance of sinus rhythm after end of exercise
		Few min	Runs min	min
Oct 20	None		Immed	2
Oct 21	1/2 hour after propranolol 10 mg		15 sec	1
	1 1/2 hour after propranolol 10 mg	2 3/4	None	Immed
Oct 22	1/2 hour after propranolol 20 mg	Immed	1 1/2	1
	1 1/2 hour after propranolol 20 mg	2 3/4	None	Immed
	4 hours after propranolol 20 mg	None	None	
Oct 23	2 1/2 hours after propranolol 20 mg	2	None	Immed
	4 1/2 hours after propranolol 20 mg	2	2 1/2	1
Oct 24	None		15 sec	2
Oct 27 1 20 p m	Propranolol 10 mg × 3 from Oct 26 Last dose at noon	1	2 1/2	30 sec
Oct 29 1 30 p m	Propranolol 10 mg × 4 daily Last dose at noon	3/4	2 1/2	15 sec
Oct 31 4 tests	Propranolol 10 mg × 4 daily (6 a m 10 a m 2 p m , 5 30 p m)			
	1 At 8 30 a m (2 1/2 hours after 1st dose)		2	25 sec
	2 At 11 00 a m (1 hour after 2nd dose)	1	2	15 sec
	3 At 1 45 p m (3 3/4 hours after 2nd dose)	1	2 1/4	15 sec
	4 At 5 00 p m (3 hours after 3rd dose)	1	2 1/2	20 sec
Dec 1 11 30 a m	Propranolol 10 mg × 4 daily (7 a m 10 a m 2 p m 5 20 p m) Last dose at 10 a m	1	2 1/2	25 sec
1965 Jan 18 12 30 p m	As above	2	2 1/4	15 sec

At no time during the whole treatment were any abnormalities noted in different blood tests (hemoglobin, complete blood count including platelets, GO transaminase serum creatinine, thymol and serum bilirubin) or urine tests (protein catecholamines)

2 PATIENT K B H Department of Medicine B record no 825/64 65) Girl

born 1956 was admitted on October 19th, for treatment of episodes of unconsciousness There was no history of congenital heart disease or epilepsy in the family The pregnancy and delivery were normal The birth weight was 3600 g Growth and development were normal and apart from mild forms of common childhood diseases she had been well until May 1962

From that time until December 1963 she had 6 attacks of sudden loss of consciousness all in connection with straining The attacks were first mistaken for epileptic seizures but after admission to the University Clinic of Paediatrics (record no 977/63) on January 4th 1964 a diagnosis of Adams Stokes syndrome was established An electrocardiogram obtained after heavy exercise showed runs of multifocal ventricular extra systoles although the resting electrocardiogram the chest roentgenogram and auscultation were entirely normal Thus no other sign of heart disease was found and heart catheterization was not indicated A detailed history up to that time has been published previously (11) She was discharged with the advice to avoid exertional strain This however seemed to be impossible for her and as she had another attack in April 1964 and one in August 1964 she was admitted for medical treatment

Treatment She entered the hospital about one month after the first mentioned patient who at that time had been treated for the same disease with different drugs which had no effect and who had just started to take propranolol This was then immediately tried on this patient also since the basic mechanism of the attacks seemed identical Trials likewise made during exercise gave an equally good result (table II) The patient was discharged on a dose of 10 mg four times daily and was seen for follow up 1 and 2 1/2 months later

Follow up During the 2 1/2 month period she had no syncopes She was however according to her mother somewhat emotionally unstable with fits of temper — but she had always been stubborn not living well with her nine year-old sister and her five year-old brother The same blood and urine tests as in our first patient were normal and exercise test on a bicycle ergometer with the usual load was unchanged (table II) According to our last information the patient is still doing well 4 months after discharge

Discussion

Stock and Dale (8) have studied the action of pronethalol (Alderlin) in digitalis induced arrhythmias and in a variety of other arrhythmias with intravenous administration of the drug Among 6 patients with very frequent extra systoles these were abolished in 4 by the time the injection was completed no further trial was reported 1 patient with atrial tachycardia was treated without success, but only one out of 3 patients with ventricular tachycardia converted to sinus rhythm on pronethalol

Grandjean and Rivier (4) likewise studied the effect of intravenously injected pronethalol in 12 patients with different cardiac arrhythmias among whom was one of auricular tachycardia due to digitalis intoxication and one of runs of ventricular extra systoles, in the former the sinus rhythm was established, while there was no effect in the latter patient

Only Ward (10) has so far been concerned with adrenergic beta receptor blocking as a treatment in Adams-Stokes syndrome due to tachyarrhythmias and his report is also the only one where the newest agent propranolol has been used in a child Ward describes

2 siblings with Adams Stokes attacks precipitated by exertion At rest the only cardiac abnormality in both was a prolonged Q-T interval The elder one, a 6-year old girl with attacks since the age of 16 months, had during the diagnostic investigation done an exercise test, during which she had an attack while the electrocardiogram showed abnormal complexes leading to ventricular fibrillation After some trial the attacks were controled with pronethalol (Alderlin), the effect of which was followed on the decrease of extra-systoles during exercise on a bicycle ergometer No side effects were seen during the next 6 months Her 17 month old brother then began to have similar attacks which could not be stopped by pronethalol he was transferred to propranolol 5 mg three times daily and had no attacks for a week He, however, developed marked irritability and received therefore in addition thioridazin (Melleril) 10 mg twice daily He remained irritable with frequent lapses into unconsciousness, and the parents withheld the treatment for a day 18 hours after his last dose of propranolol he died in a syncope

The two patients described in our paper had wihtout doubt Adams Stokes syndrome due to long runs of ventricular extra systoles, possibly leading to other tachyarrhythmias Only in one of the patients has an electrocardiogram been obtained during the last part of an attack, showing paroxysmal ventricular tachycardia and multifocal extrasystoles but the persistent appearance of multifocal extra systoles during exercise together with the fact that the attacks were provoked by exertion should be enough proof of the mechanism (11) No organic heart disease was found in either of our two patients, the etiology in the first mentioned was probably a myocarditis, while it is unknown in the other one Nor was any Q T abnormality present, or any history of heart disease in the family

The ready appearance of extra systoles during exercise gave us the opportunity to evaluate the anti arrhythmic effect of propranolol in both patients, in one of them it was shown in addition that the same effect could not be obtained by drugs as quinidine, procainamide, antazoline, ajmalin and phenytoin The effect was judged by the time it lasted before extra-systoles appeared after begin of exercise and by the length of time required for sinus rhythm to reappear after exercise Although no complete suppression of the arrhythmia was achieved, the effect is obvious (tables I and II) Especially noteworthy is the reversed effect on October 24th, in both patients, when propranolol was discontinued

As shown in the tables the effect was demonstrable within 1 1/2—4 hours after propranolol given orally while no effect was seen 1/2 hour after the dose and none 6 hours after indicating an insufficient blood concentration This is in accordance with available information from the research department of the manufacturer Consequently we have instructed our patients to take their first dose fasting as soon as they wake so that a sufficient blood level has been reached when they start for school one hour later, and no more than 4 hours

must elapse between the doses during their "out of bed" time

With regard to the side effects of propranolol, dizziness, nausea and anorexia have been reported in the treatment of angina of effort (7) and tiredness, depression, giddiness and visual hallucination have been encountered in the treatment of hypertension (5), both reports dealing with adults receiving higher doses than used by us Recently biochemical changes have been observed that suggest hepatic or renal damage in 4 patients to whom propranolol was given in an attempt to maintain sinus rhythm after electrical defibrillation (9)

During the follow up drowsiness appeared in one of our patients so that the dosage had to be reduced to a lower and less effective level No other side effects were observed and no biochemical changes were seen The emotional instability in the other patient was probably due to domestic problems and was maximal during the first time after her dischange from the hospital where she had been exposed to a great deal of attention

The ultimate effect and the side effects of propranolol in long term treatment have still to be evaluated The prognosis in our two patients is considered serious unless the attacks are stopped (11) and we have held it justified to use a drug which — though rather new and unknown — so far has prooved effective at least in one patient

Summary

Two children with Adams Stokes syndrome due to tachyarrhythmia during exercise were treated with a new adrenergic beta receptor antagonist, propranolol (Inderal)

The effect was judged from electrocardiograms during and after exercise on a bicycle ergometer, during treatment the arrhythmia was not suppressed, but a longer time elapsed before the arrhythmia appeared after the start of exercise and sinus rhythm was reestablished sooner after the exercise

In the course of 4 months one patient had no Adams Stokes attacks The other patient had 2 slight attacks and also became tired and drowsy so that her dose had to be reduced to a less effective level No other side effects were encountered

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Circumcision and Prostatic Cancer

By

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The present paper will analyse the occurrence of genital carcinoma in people who practise ritual Mosaic circumcision versus uncircumcised populations with special regard to prostatic carcinoma which is the top ranking carcinoma in Swedish males

The technique of circumcision

Ritual circumcision is practised by the Hebrews when the child is 8 days old unless medical contraindications exist The procedure is that the foreskin is drawn forward in front of the glans and cut off To avoid injury to the glans the foreskin is drawn through a slot in a metal plate and the cut is made along the plate The operation is actually an amputation which would have been a more correct expression than circumcision After the cut has been made the outer preputial layer is drawn back behind the glans The adherences of the inner layer are released and the layer is folded back after which it is joined to the outer layer The preputial remnants heal within only a few days The operation is performed by a specially trained *mohel* who in occidental countries is usually an ordinary surgeon

The Mohammedans have a somewhat different procedure The child is rather older and the technique differs in some respects The age of circumcision varies in different places usually between 5 and 15 years of age In Palestine it is usually done at the age of 13 years as Ismael the progenitor of the Arabs was circumcised at that age The technique differs from the Mosaic in that when the foreskin has been drawn forward and cut off the operation is complete The two preputial layers thereafter grow together at roughly half the length of the glans so leaving a small preputial pocket This has its consequences of which more will be said later

Immediate and remote advantages of circumcision

The immediate advantage of circumcision particularly if performed the Mosaic way, is the elimination of the preputial space Such a person will accordingly never have to suffer from conditions such as phimosis paraphimosis pseudophimosis and balanitis These conditions are otherwise by no means uncommon Bonnevier (3) states that pseudophimosis occurs in 2 9—20 %, real phimosis in 1 2—10 % in various

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investigations, estimating an average occurrence of 5 % in both conditions Balanitis will ensue particularly in phimosis, connected with bad hygiene

The remote advantages of circumcision are associated with balanitis as disposing factor for carcinoma It has been maintained that if the male partner has been subjected to circumcision penile carcinoma will be less common, as well as cervical and uterine carcinomas in the wives of such men Are there still other carcinomas for which circumcision may be of prophylactic value?

1 Penile carcinoma

Penile carcinoma occurs practically not at all in persons circumcised by the Mosaic ritual A study by Wolbarst appears to be particularly instructive He writes "Not a single case of penile cancer in a circumcised Jew has been reported in a total of 1,103 cases in the United States, despite the fact that Jews constitute about 3 per cent of the American population" A single case of penile cancer was reported in a Jew, but this man had not been circumcised

Other studies indicate a certain, even if low, incidence of penile cancer in Hebrews Licklider (15), in a series from the Memorial Hospital for Cancer and Allied Diseases, found that only 3 3 per cent of the cases occurred in Jews though the latter represented 30 per cent of all patients It is not clear, however, how many of the Jews and non Jews had been circumcised In America there are many assimilated Jews who are incircumcised and many non Jews who are circumcised Comparisons between Jews and non-Jews in America are generally of doubtful value as increasing numbers of Americans have their sons circumcised During a visit to the United States in the spring of 1964 Ask Upmark learned that the number of circumcised non Jews was already considerable and was growing Among elderly non Jews there are also many circumcised Gibson (7) reported on a material in which 20 per cent of the non-Jews were circumcised Figures from the United States are therefore liable to be misleading as they refer to Jews and non-Jews instead of circumcised and uncircumcised

Higginson and Oettle (9) consider that circumcision during the years of growth does not provide the same protection against penile carcinoma as when it is done in infancy For this reason penile carcinoma is commoner among Mohammedans than among Jews In this respect the different technique, by which the Mohammedan leaves more of the foreskin, is also of significance Among uncircumcised peoples the incidence varies very greatly According to Bonnevier (3), in Indonesia penile carcinoma represents 37 8 per cent of all male carcinoma, in China 27 9, in India 10 per cent In India, as expected, it is much less common among the circumcised Mohammedans than among the remainder of the population Wolbarst states that among 1,336 penile cancers in India 1 302 occurred in Hindus (97 5 %) and 26 in Mohammedans (2 %), whilst the average Mohammedan population was 21 19 % In Uganda (5) penile carcinoma is the most common form of male carcinoma, amounting to 12 2 per cent Circumcision is practised only within one tribe in Uganda the

Gisus and within that tribe only a single case was found

The cause of penile carcinoma is the content of the preputial pocket, the smegma and the infectious content Hofmeister (10) reports phimosis to exist in 90 per cent of cases of penile cancer In persons with a low standard of personal hygiene it is more common It is therefore very much rarer in the West than among underdeveloped peoples In Sweden (18), in 1960 there were only 15 cases of cancer of the penis, scrotum and other male genital organs (apart from cancer of the prostate and testis)

2 Cervical and uterine carcinoma

Even if penile carcinoma is no great problem cervical carcinoma is all the more so In Sweden there were 779 cases in 1960 7 2 per cent of all cases of carcinoma in women It is thus one of the most common forms of carcinoma Investigations in the United States and in Israel have directed attention to the relation between this form of carcinoma and the state of circumcision or non circumcision in men Statistics of the cancer mortality in Israel from 1950 to 1957 have been published by Kallner (11) According to the latter the proportion of cervical to all cancer cases is in England and Wales (1952) 4 9 % in Holland (1952) 2 2 % in U S A (1952) 3 7 % and in Israel 0 5 % The number of cervical cancer cases per 100 000 women is shown in table I These figures suggest that there is a very definite difference in the incidence of cervical cancer among Jewesses and non Jewesses Later statistics, however, show a very much less pronounced difference of which more below

TABLE I Incidence of cervical cancer per 100 000 women

Age group 45—54 years		65 years and above
England and Wales (1952)	17 3	32 3
Holland (1952)	16 1	23 3
Israel (1950—1954)	2 3	3 9

Dunham et al (4) have reported statistics of uterine cancer among different population groups in New York The incidence among Jewesses was the same as in Israel and barely one third of that among white non Jewesses In negresses the figure was three times as high as in white non Jewesses and among Puerto Ricans more than twice as common as among negresses The authors do not consider it proved how ever that circumcision is the decisive factor in the difference of incidence among Jewesses and non Jewesses They point out that there are many other factors one being that women who have sexual relations with several men are more prone to uterine cancer Rotkin (20) has provided evidence that cervical cancer is more common among women who started their sexual life early Nuns and others who live in sexual abstinence practically never have this cancer Handley s observations from the Fiji Islands (8) are instructive Among 90 000 Fijians (men circumcised) 3 cases of cervical cancer were discovered among 70 000 Hindus (men uncircumcised) 26 cases These two populations live side by side but do not intermingle

TABLE II Incidence of cancer per 100,000 women

	Age group					
	25—34	35—44	45—54	55—64	65—74	75+
Sweden	15	46	43	34	19	15
Israel	1	10	13	19	19	0

A decisive factor in cervical carcinoma is personal hygiene, not least in the male sexual partner In Uganda (5), where penile carcinoma is the most common form of carcinoma in men, cervical carcinoma is the most common form in women Figures from New York (excepting Jewesses) show that cervical cancer is more common the lower the social standard of the polulation group In England (6) the incidence of cervical carcinoma among doctors wives, lawyers' wives and clergymen's wives is barely one fifth that in the remainder of the population The causative factor in cervical carcinoma is the same as in penile — the smegma and balanitis, which give rise to chronic cervicitis The smegma in itself has a cancerogenic effect The incidence of cervical carcinoma is lower when a condom is used as preventative (6, 22)

Comparisons between Jewesses and non Jewesses (4) may be difficult to evaluate since, as already stated, many non-Jews are circumcised while some Jews are not One knows, too, that the possibility of medical and hospital care in America is greatly dependent on the individual's income For comparison between two groups, in one of which practically all are circumcised and in the other practically none, and in both of which the availability of medical attention and hospital treatment is unrelated to the individual's finances, we may consider the incidence of cervical cancer in Israel and in Sweden The figures are taken from the Cancer Registry of each country, which for Sweden relates to 1960 and for Israel to 1960 and 1961

Reference to the differing conditions in Sweden and Israel will be made later In the preface to the Israeli Cancer Registry it is pointed out that the earlier very satisfactory figures of cervical cancer cannot be confirmed, also that one should not count on the Arab women since they object to visiting a doctor for genital diseases The Cancer Registry gives the incidences in the different population groups and in the sequel we shall consider Jewesses alone Cases indicated as of unknown origin will be counted as Jewesses This group may thus be said to include rather more cases than it should, but at least does not include too few

In Israel there were at the end of 1961 (29) 1,100,865 women, 1,004,212 of whom were Jewesses The number of cases of cervical cancer among the latter was 54 in 1960 and 16 in 1961, on an

average 50 per million women According to the Swedish Cancer Registry there were 779 cases in Sweden, i e 208 per million women

Now cancer is, of course, a disease associated with certain ages The age distribution in Sweden differs greatly from that in Israel This will be considered later A comparison of the incidence within the different age groups per 100,000 women is shown in table II The proportion is 4 1 at the ages in which cervical cancer is most common, 35—54 years and the disease starts at earlier ages and continues until higher ages in Sweden than in Israel

3 Carcinoma of the corpus uteri

Whereas a *comparison* between the incidence of cervical carcinoma in populations in which circumcision is generally practised and those in which it is not gives compatible results, the circumstances as regards carcinoma of the corpus are less clear Whereas the aforementioned study from New York (4) showed a distinctly lower incidence among Jewesses and a falling incidence *in* the order Puerto Ricans negresses white non Jewesses Jewesses, the figures for carcinoma of the corpus were almost ly exactly reversed, namely Jewesses in New York white non Jewesses, negresses Puerto Ricans and — the lowest incidence — Jewesses in Israel The differences as regards carcinoma of the *corpus* are to be sure not so great as in respect of cervical carcinoma The ratio of the largest group Jewesses *in* New York to the smallest Jewesses in Israel is 3 1 The situation is nevertheless difficult to understand Why should carcinoma of the corpus be three times as common in the former as in the latter? Is it possible that the term *carcinoma* of the corpus may be differently defined?

If, for the reasons stated earlier, we instead compare the incidence of carcinoma of the corpus in Sweden and among Jewesses in Israel, we find that in Sweden in 1960 there were 606 cases, i e 162 per million women in Israel there were 100 cases among Jewesses and women of 'unknown origin' in 1960 86 in 1961, average 93 per million This figure is undoubtedly too high, however, as the 'unknown origin' group includes 14 cases in 1960 and 13 in 1961, several of whom were probably not Jewesses The Cancer Registries of the two countries thus indicate that the incidence of carcinoma of the corpus is nearly twice as high in Sweden as among Jewesses in Israel

In discussing the incidence of genital carcinoma among Jewesses it would be interesting to have a comparison between orthodox Jewesses and *assimilated* groups There is reason to believe that the strict sexual morality and the rigorous sexual hygiene on religious precepts among the former have a significance as cancer prophylaxis I have not discovered any such report It would also be interesting to have *figures of the* incidence of cervicitis leukorrhoea and similar states which undoubtedly predispose to cancer among orthodox Jewesses assimilated and non Jewesses This might explain the different findings in respect of the incidence of carcinoma of the corpus among different *Jewish* groups

TABLE III Deaths of Hebrew males in Scandinavia

	Total	Deaths from carcinoma	Deaths from prostatic carcinoma
Stockholm	785	134	4
Gothenburg	171	23	2
Malmo	152	27	3
Copenhagen	427	77	3
Total	1 535	261	12

TABLE IV Cancer and prostatic cancer as causes of death

	Cancer as cause of death	Prostatic cancer as cause of death	Percentage of prostatic cancer in cancer deaths
Sweden	16 6 %	1 9 %	11 8 %
Scandinavian Jews	17 0 %	0 78 %	4 6 %

4 Prostatic carcinoma

May there be reason to suppose that circumcision is a prophylactic against this form of cancer, the most common male form in Sweden? According to Ask-Upmark (1), who suggested that I carry out the present study, there seems to be reasonable evidence that prostatitis is the underlying lesion in several instances of prostatic carcinoma The occurrence of prostatitis, on the other hand, seems to be favoured by phimosis and balanitis

Some statistics, but not others, have earlier shown a lower incidence of prostatic carcinoma among Jews than among others Whether this was true has been discussed in the Anglo-American literature for some time past Ravich (16) and Ravich and Ravich (17) found 2 per cent prostatic carcinoma among their circumcised patients against 20 per cent among uncircumcised Wilhelm (24) found 5 per cent prostatic carcinoma among the Jewish cases of cancer Robb and Roemmele (19), on the other hand, found no difference in the incidence of prostatic carcinoma between Jewish and non-Jewish patients Gibson (7) came to the same conclusion In his series 20 per cent of the non Jews had been circumcised in infancy

These contradictory results can be explained, at least to some extent, by the comparison of different concepts Some have compared Jews and non Jews, others circumcised and uncircumcised Other statistics relate to different hospitals, not to entire populations. Figures from America are not generally clear in view of the very large group of non Jews who are circumcised Another source of obscurity is that authors seldom state what they mean by prostatic carcinoma Apart from the clinically malignant form there is a clinically benign but histologically cancerous form To obtain comparable figures one would need two groups, one consisting exclusively of uncircumcised and the other exclusively of circumcised men but similar in their living conditions in their social environment in their access to medical attention and hospitals and in their average length of life (an important point as prostatic carcinoma is to a large extent a disease associated with advanced age) If one then keeps merely to the causes of death, the different definitions of prostatic carcinoma are of

no importance Two such groups are the Scandinavian Jews compared with the remaining Scandinavian populations I have therefore gone through the registers of deaths and burials of the Mosaic congregations of Stockholm, Gothenburg, Malmo and Copenhagen (I did not visit Norway, as there are very few Jews there For the sake of brevity however I shall speak of Scandinavian instead of Swedish and Danish Jews) The material covers the years 1946—1963 for the Swedish and 1949—1963 for the Danish congregations, in the latter case older statistics were not available The years before 1946 in the Swedish registers have not been included as the war year statistics were altogether abnormal on account of the refugees who came to Sweden during the war and of the large number of persons from concentration camps who were brought to Sweden in 1945 many of whom died soon after their arrival of cachexia tuberculosis typhus etc As from 1946 the conditions became normal again

By cancer is meant in the sequel diseases listed in the Swedish Cancer Registry The figures relate only to men "Deaths do not include stillborn infants

For deaths of Hebrew males in Scandinavia see table III

If we take for our comparison one year between 1946 and 1963, say 1955 the causes of male deaths are given by the Central Bureau of Statistics as total deaths 35 542 deaths from cancer 5 855 deaths from prostatic cancer 692 Comparing these figures with those of the Jewish population (table III) the results are as shown in table IV

TABLE V Number of histological diagnoses in the various cancer groups in Sweden and Israel

	Sweden	Israel
Cancer prostatae	62 8 %	56 %
Cancer cervicis	99 5 %	90 %
Cancer corporis uteri	100 %	100 %

The cancer mortality is thus roughly the same among Jews and the remainder of the population but the prostatic cancer mortality is considerably lower

It has sometimes been questioned whether necropsies are carried out with equal frequency among orthodox Hebrews as among other people Once in a while objections may be raised by the relatives but the same is the case with many non Hebrews The Hebrews never object to medical examination surgical operation or other treatment for religious reasons The reports of the Department of Pathology of the University of Jerusalem are good evidence of the frequencey of necropsies among the Hebrews Moreover should permission for a necropsy occasionally not be granted, why should this result in a less frequent diagnosis of prostatic than of other carcinomas? The overall mortality in carcinoma is the same in Hebrews and non Hebrews

On the other hand one may well object that the series is too small To obtain a larger series, and to study the conditions on the spot, I made a voyage to Israel in 1964 For in order correctly to compare statistics from Israel and Sweden, one must take into account

some quite considerable differences between the two countries

I have referred above to the objection to a Hebrew material on grounds of the alleged Hebrew dislike of necropsies I have therefore compared the number of histological diagnoses for the relevant cancer groups within the two countries (table V) The figures are taken from the Cancer Registries The difference is not so large as to be of any decisive importance

To obtain a comparison between circumcised and uncircumcised, one group should comprise only the former and the other only the latter In Sweden practically all males are uncircumcised What is the situation in Israel? Israel has many Jewish atheists, but the various people I consulted were unanimous that the latter also practise circumcision on a 100 per cent scale

The population of Israel at the year end 1961 was 2,234,209 (29), of whom 1,133,338 males and 1,100,212 females Of the males 1,004,986 were Jews, 89,986 Mohammedans, 25,412 Christians, and 13,728 Druses Only 2 2 % (the Christians) were uncircumcised, 88 7 % circumcised by the Mosaic and 9 1 % by the Mohammedan ritual On the whole, therefore, one may speak of a circumcised population

Another question is whether the medical standards are comparable, the relative availability of physicians and hospitals, and whether there are financial impediments to visiting a physician and hospital On these points I may say that medicine in Israel is not on a lower level than in Sweden, that there are relatively twice as many doctors in Israel as in Sweden, that the hospital system is highly developed, and that there are no financial hindrances to medical attention and hospital treatment The number of hospital beds is relatively lower in Israel, but the outpatient departments have a very much greater significance there than in Sweden

Then there is the important question whether certain groups — Arabs, oriental Jews etc — visit a doctor to the same extent as occidental Jews As regards Arab women it has already been noted that they shun visiting a doctor for diseases of the genital organs and I have therefore excluded them from the discussion of uterine cancer This does not apply, however, to male Arabs The situation as regards oriental Jews (Sephardim) is questionable N Kook and H Kook (14) consider that the orientals present to a doctor to a lesser extent Among 542 patients above 45 years of age who presented for urination complaints they found 9 cases of prostatic cancer, 8 of them Ashkenazim (occidentals) and 1 Sephardi Of 3,068 cases of hypertrophy of the prostate in Israeli hospitals from 1956—1958 there were 168 cases of prostatic cancer, 129 of them Ashkenazim and only 39 Sephardim But it is not stated how many of each group had presented at hospital According to Kallner (11) there were, on the contrary, more orientals than occidentals among the cases of prostatic cancer, 2 7 against 2 3 per cent

A very important question when comparing so pronounced an age related disease as prostatic cancer in two populations is their relative age distribution This differs greatly between the two

countries Owing to her abnormally low birthrate Sweden has an age group distribution with the graphical shape of an urn In Israel with her large immigration of young people and relatively high birthrate, a graphic representation of the age groups takes the shape of a pyramid which rapidly narrows upwards The age group distribution for males in 1961 is shown in table VI

According to the two Cancer Registries the number of cases of cancer per 1 million males in Sweden was 2,589 and in Israel 1 894 If one took no account of the relative age distribution one might draw the conclusion that cancer is more frequent in Sweden In actual fact the reverse applies But a study of the incidence of cancer in the real cancer ages shows the following figures (table VII) in which the number of cases is given per million males for each age group This shows that cancer is commoner in Israel than in Sweden There appear to be no grounds for the suspicion that the data in the Israeli Cancer Registry are incomplete

Proceeding now to the data of prostatic cancer in the Cancer Registries we find that the number of cases in Sweden was 1 544 in Israel 88 in 1960 109 in 1961 *The annual rate in Sweden is 414 per million males in Israel 88* This suggests a very great difference between the two countries But as prostatic cancer occurs typically within certain age groups one must look at the relative age distribution in these groups Table VIII shows the number of cases of prostatic cancer per million males in the age groups in which it is most common (absolute figures in brackets)

TABLE VI The distribution of males in various ages

Age	Sweden	Israel
0— 4	7 1	12 4
5— 9	7 3	12 3
10—14	8.2	11 8
15—19	8 3	8.9
20—24	6 4	6 7
25—29	5 9	6 6
30—34	6 4	6 0
35—39	7 0	6 0
40—44	7 3	5 4
45—49	7 1	5 4
50—54	7 0	5 7
55—59	6 1	4 5
60—64	5 0	3 3
65—69	4 1	2 1
70—74	3 0	1 5
75 and above	3 9	1 4

TABLE VII Cancer incidence in the real cancer ages. *In each group the number of cancer cases is calculated per million males*

Age	45—54	55—64	65—74	75 and above
Sweden	1 834	5 124	12 110	17 903
Israel	3 058	6 794	13 132	18 186

TABLE VIII Calculated number of cases of prostatic cancer per million males (absolute figures in brackets)

	Age group 65—74	75 and above
Sweden	2 436 (647)	4 919 (655)
Israel	1 014 (82)	2 133 (64)
Ratio Sweden Israel	2 3 1	2 3 1

TABLE IX Mortality in cancer and prostatic cancer in Sweden and Israel

	Total deaths	Cancer deaths	Prostatic cancer deaths
Sweden 1960	39 511	7,132	1,003
Israel 1960—61	11,574	2,280	116

Prostatic cancer is thus 4 7 times more frequent in Sweden than in Israel, but in the ages in which it is most common only 2 3 times The statistical material is so large that there can be no question of mere chance

A comparison of the mortality in males is shown in table IX

Table X shows the relationships between these figures and the previously mentioned figures for Sweden in 1955 and Scandinavian Jews 1946—1963

Prostatic cancer appears to have increased considerably in Sweden in recent years The figures for Israelis and Scandinavian Jews are closely similar As regards all forms of cancer the mortality is rather higher among Jews, but in prostatic cancer considerably lower The mean share of prostatic cancer in the mortality is 2 2 per cent in Sweden against 1 0 per cent in Israel, which agrees well with the incidence of prostatic cancer in the higher ages, in which the ratio was 2 3 1

Earlier reports from Israel (11) likewise show the share of prostatic cancer in cancer mortality to have been 5 %

To sum up, it may be said that penile cancer occurs practically not at all among circumcised Jews, but is of little significance in Sweden either Prostatic cancer, the commonest form of all male cancer in Sweden, is less than half as frequent among the circumcised, cervical cancer is four times less common and cancer of the corpus less than half as frequent in Israel as in Sweden The fact that genital cancer occurs so very much less among Jews, while other forms of cancer are rather more common among them, cannot be due be to anything else than circumcision *Through universal circumcision some 1,800 cases of cancer a year would be avoided in Sweden on the basis of the above figures*

Opinions concerning universal circumcision, however, vary considerably Ask-Upmark (2) considers that circumcision by the Mosaic ritual should be compulsory Dodge & Linsell (5) write concern

TABLE X Share of cancer and prostatic cancer in total mortality in Sweden and Israel

	Percentage of cancer in total mortality	Percentage of prostatic cancer in total mortality	Percentage of prostatic cancer in cancer mortality
Sweden 1955	16 6 %	1 9 %	11 8 %
Sweden 1960	18 0 %	2 5 %	14 0 %
Israel 1960—1961	19 7 %	1 0 %	5 0 %
Scandinavian Jews 1946—1963	17 0 %	0 78 %	4 6 %

ing penile carcinoma in Uganda "Carcinoma of the penis is perhaps the most easily preventible form of cancer It may not be easy to persuade a whole country to adopt an unfamiliar and perhaps repugnant practice to achieve so apparently remote a benefit" This statement relates to the natives of Uganda but applies also *to other countries* Bonnevier (3) does not regard a phimosis incidence of 5 per cent as motivating universal circumcision He considers it of great significance in populations with a low standard of hygiene but in Sweden — with its excellent hygiene and well developed information services — he does not think universal circumcision warranted from the point of view of cancer prophylaxis Karlstrom (13) is of of the same opinion

I cannot adhere however, to this opinion that a high standard of hygiene renders circumcision unnecessary Hygiene in Sweden is undoubtedly no lower than in Israel especially when one thinks of her oriental and nomad populations but genital cancer is nevertheless very much more common in Sweden This applies particularly to prostatic cancer which was not dealt with by the latter authors (3, 13) I therefore consider that universal circumcision must be acknowledged to have a definite value from the point of view of cancer prophylaxis

Summary and conclusions

1 The author has studied the occurrence of genital carcinomas in Israel as compared with Sweden The medical facilities of the two countries are comparable, as are the economic conditions

2 With regard to carcinoma of the cervix the author finds the occurrence in Sweden to be at least 4 times as high as in Israel

3 As to carcinoma of *corpus uteri* its occurrence in Sweden is about twice as common as in Hebrews in Israel

4 Prostatic carcinoma was the main topic of the present study and a special investigation was made between, on the one hand, Israel and Scandinavian Jews (circumcised) and on the other the Swedish population in general (uncircumcised) The study was based on statistical analyses of the cancer material as recorded in Israel and in Sweden as well as in the Jewish communities in Denmark and Sweden Prostatic carcinoma is less than half as frequent in circumcised as in uncircumcised populations

5 Circumcision seems to be the essential factor in preventing not only several instances of female genital carcinomas but also carcinoma of the prostate Since this tumour is the top ranking carcinoma among males in Sweden it seems reasonable to suggest circumcision along the lines indicated by the Mosaic rite as carried out already on a wide scale in the United States

6 When these questions are discussed one should distinguish not between Jews and non Jews but between peoples who practise circumcision and those who do not This distinction has not always been observed in the literature

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Phenylbutazone and Leukaemia

By

MOGENS KROGH JENSEN and KAI ROLL

Since 1958 several reports of leukaemia associated with the administration of phenylbutazone (PBZ) have appeared (2, 4 5 6 7 11, 13 24, 28 31) With the purpose of elucidating the question of the possible leukaemogenic action of PBZ we have reviewed the pertinent literature and performed a retrospective survey of patients with acute leukaemia with respect to PBZ exposure

From 1959 to 1964 fifty patients with acute leukaemia were admitted to Medical Department A Rigshospitalet Three of these patients had a history of previous ingestion of PBZ

Case reports

Case 1 A 78-year old author was admitted with symptoms of anaemia For three years he had suffered from pains in both wrists and proximal joints of the thumbs. In June 1960 treatment with phenylbutazone 600 mg daily was started Favourable effect on the joint pains was observed and the treatment was continued for four months at the same dose

In September 1960 the patient began to complain of fatigue and weakness From late October 1960 until June 1961, he was admitted four times to another hospital In October 1960 a pancytopenia was found Hb 5 8 g % W B C 2 080/μl with normal distribution Platelets 102 000/μl Marrow aspirates in October 1960 January, and June 1961 were hypocellular with a shift to the left of the myeloid precursors erythropoiesis was absent The condition was interpreted as an aplastic anaemia or an subacute or chronic myelocytic leukaemia with pancytopenia During each admission the patient was given 6—8 transfusions Furthermore he was treated with prednisone 15—30 mg daily without any significant effect on the anaemia

Having had a fit of unconsciousness during a trip to Italy the patient was transferred from an Italian hospital to this department in September 1961 At admission the patient was found very pale and dyspnoeic Oedemas of the ankles were noted Apart from slightly enlarged lymph nodes in both axillas no swollen lymph nodes could be felt The liver and spleen were not palpable There was no haemorrhagic diathesis and the temperature was normal Hb 6 9 g % Reticulocytes 0 ‰ W B C 6 200/μl Eosinophilia (9 %) and basophilia (4 %) was noted but otherwise the differential count was unremarkable The smear showed slight anisocytosis of the red cells Thrombocytes

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14,000/μl Serum iron 193 μg % Transferrin 200 μg % MCV 111 μ^3 MCHC 33 g % Haptoglobin 280 mg % Serum bilirubin 0 2 mg % Coombs' test negative E S R 68 mm/h A streaky haemorrhage of the fundus of the left eye was found The bone marrow was highly cellular with a shift to the left of the myeloid cells Erythropoiesis was scanty A diagnosis of subacute or chronic myeloid leukaemia was made and treatment with 6 mercaptopurine was commenced In addition the patient received four units of blood

The patient was readmitted late September, 1961, suffering from increasing fatigue, dizziness and dyspnoea Hb 8 6 g % W B C 11,100/μl with 9 % eosinophils but otherwise the differential count was normal Thrombocytes 77,000/μl He was given three transfusions and discharged with prednisone 7 5 mg daily

In the middle of November, 1961, the patient was admitted to another hospital after a period with fever, haemorrhagic diathesis and increasing symptoms of anaemia Hb 9 1 g % (four weeks previously eight transfusions were given during an admission) W B C 24,800—68,800/μl with 14 % blasts, 8 % myelocytes, 4 % metamyelocytes, 36 % juvenile neutrophils, 18 % segmented neutrophils 14 % lymphocytes, and 6 % monocytes There were 0—6 normoblasts per 100 nucleated cells in the peripheral blood Thrombocytes 6,000—37,000/μl The patient s condition deteriorated rapidly and death ensued at the beginning of December, 1961, in cardiac failure

At autopsy pulmonary oedema and haemorrhage of the renal pelves were found The spleen weighed 760 grs The liver measured 7 × 21 × 26 cm Histopathological examination showed a hyperplastic marrow which was dominated by myeloblasts Diffuse myeloblastic infiltration of liver, spleen kidneys, lungs muscles, and around the pituitary gland was found

Case 2 A 67-year old driver For six years he had been suffering from rheumatoid arthritis After the patient had received phenylbutazone 300 mg daily for a few months with good effect on the joint pains, he was admitted to another hospital in March, 1961, on account of gastrointestinal haemorrhage The patient was pale but not in shock Arthritic deformities of the joints were noted Hb 8 6 g % W B C 4,900/μl with normal distribution E S R 127 mm/h Roentgenographic studies of the oesophagus and the stomach showed neither varicose veins nor ulcers Liver function tests were normal It was believed that the gastro-intestinal haemorrhage was provoked by the ingestion of phenylbutazone, and he was treated with an ulcer diet

The following two years the patient was well without dyspepsia or joint pains In April, 1963, he was admitted to this department because of gastro-intestinal haemorrhage For three weeks he had suffered from increasing fatigue, dizziness and dyspnoea At admission the patient was pale and febrile There was no haemorrhagic diathesis of the skin and oral mucosa The lymph nodes were not enlarged, and the liver and spleen were not palpable Hb 5 7 g % W B C 1,800—3,600/μl with 28 % myeloblasts in the peripheral blood Thrombocytes 80 000/μl Haptoglobin 602 mg % Serum bilirubin 1 5 mg % Reticulocytes 0—2 ‰ Coombs' test negative E S R 168 mm/h Rheumatoid Arthritis Test + + + Rose Waaler s test positive Ophthalmoscopy showed haemorrhages and a small exudate of the right fundus The marrow was hyperplastic, dominated by myeloblasts Erythroid precursors were scanty The patient was treated with 6-mercaptopurine 150 mg daily He developed a bronchopneumonia and septicaemia and despite treatment with antibiotics death occurred early in May 1963

At autopsy bronchopneumonia and a right sided pulmonary abscess were found The bone marrow was hyperplastic dominated by blast cells but no infiltration of the organs with blast cells was seen

Case 3 A 31 year old female dressmaker Since July, 1956 she had been suffering from severe shooting pains in both arms and

legs In January 1957, the patient was admitted to another hospital because of the pains in the extremities An E S R of 70 mm/h was found Hb 8 0 g % W B C 6 200/µl with a normal distribution Antistreptolysin titre and antistreptococcyaluronidase titre normal Streptococcus-agglutination reaction positive The left knee was swollen Roentgenographic studies of the skull chest, spine pelvis, and knee joints were normal The condition was interpreted as a rheumatoid arthritis and for ten days the patient was treated with PBZ 400—600 mg daily Repeated courses of PBZ were given during the following six months without any effect on the pains

Early in March 1958 the patient was admitted to another hospital on account of increasing fatigue and fever Numerous lymphoblasts were found in the peripheral blood and the hyperplastic bone marrow was dominated by lymphoblasts The patient was transferred to this department

On admission the patient was pale There were no swollen lymph nodes nor were the liver and spleen palpable There was no haemorrhagic diathesis Hb 8 3 g % W B C 8 900/µl with 70 % lymphoblasts in the peripheral blood The patient was treated with 6 mercaptopurine 150 mg daily and prednisone 40—10 mg daily A remission was obtained for about four months The patient died in March 1959 from septicaemia and cardiac failure

At autopsy pulmonary oedema was found The bone marrow was hyperplastic and dominated by blast cells The liver and spleen were infiltrated by blast cells

Comments

In relation to a possible leukaemogenic effect of PBZ several factors must be taken into account viz the duration of the interval from beginning of therapy to development of leukaemia (latent interval) the total dose of PBZ given and the reason for institution of therapy with PBZ

In our three patients the latent interval was 15, 27, and 12 months respectively In radiation leukaemia the lower limit of the latent period appears to be about two years If this limit applies also to other types of leukaemia induction, only one of our cases would qualify as possibly drug induced

In the first two cases the total doses of PBZ given were 72 and 27 g respectively, which should be considered fairly high The exact amount of PBZ administered to our third patient is not known, but in all probability it was considerably lower than those in the two first cases

Rheumatic pains may be an early symptom of leukaemia, particularily in children This possibility, therefore, has to be considered in every case of leukaemia developing after ingestion of PBZ Our first patient had suffered from pains in both wrists and proximal joints of the thumbs for four years before the leukaemia became manifest In view of the long duration it is hardly conceivable that the joint pains could be related to leukaemia The same applies to our second patient who during eight years had had a clinically and serologically typical rheumatoid arthritis before the development of leukaemia The third patient (the only young person among our three patients) had suffered from severe pains of the extremities for nineteen months before the leukaemia was diagnosed Apart from a swollen knee her joints were normal Roentgenographic studies of the skull chest spine pelvis, and kneejoints were normal She had an elevated sedimentation rate and a posi-

TABLE I Previous reports of leukaemia associated with the administration of PBZ and present series

Year	Author	Age	Sex	Indication for treatment	Duration of therapy	Total dose PBZ (g)
1958	Scheuer Karpin (24)	39	Female	Spond	?	?
1960	Bean (2)	69	Male	Spond	3 weeks	8 2
1960	Bean (2)	67	Male	Spond	4 years intermitt	?
1960	Bean (2)	70	Male	Gun shot seqv	5 months	Abt 25
1960	Bean (2)	80	Male	O A spond	Several months	?
1960	Bean (2)	66	Male	Gun shot seqv	4 years	Abt 150
1960	Bean (2)	63	Male	O.A	Several months	?
1961	Cast (5)	59	Female	Arthr	$2^1/_2$ years intermitt	210
1961	Garrett (11)	64	Female	Arthr	2—3 months	15—20
1962	Cadman & Lamont (4)	71	Male	Lumbago	6 days	4
1964	Thorpe (28)	56	Female	Ac arthr	17 days	5 1
1964	Hart (13)	58	Male	Back pains	2 times 7 days	5 6
1964	Chalmers & McCarthy (6)	44	Female	R A	$3^1/_2$ years intermitt	220
1964	Woodliff & Dougan (31)	78	Male	Arthr	4 years intermitt	Several 100
1964	Woodliff & Dougan (31)	53	Female	Arthr	6 years intermitt	Several 100
1964	Woodliff & Dougan (31)	81	Male	Arthr	4 years	300
1964	Woodliff & Dougan (31)	80	Female	Arthr	About 2 years	About 100
1964	Woodliff & Dougan (31)	58	Female	Arthr	5 years intermitt	?
1964	Chatterjea (7)	46	Male	?	?	?
1964	Present series	78	Male	Arthr	4 months	72
1964	Present series	67	Male	R A	Several months	About 27
1964	Present series	31	Female	R A ?	6 months intermitt	?

Latent period	Bone marrow	Diagnosis	Autopsy	Comments
?	Hypocellular	AML	+	No information about radiotherapy
20 months	(17 months before the diagn. was confirmed) normal	CML	+	No radiotherapy given
4 years	Invaded by atypical immature lymphocyt	ALL	–	No information about radiotherapy
5 months	Large mass of malign. anaplastic tumour cells	R S ?	–	No information about radiotherapy
About 1 year	–	CML	+	No information about radiotherapy
4 years	Normal	L S ?	–	No information about radiotherapy
About 18 months	Shift to the left of both erythro- and myelopoiesis	CML	+	No information about radiotherapy
32 months	Hyperplastic mainly immature cells without nucleoli and blast cells. Little erythropoiesis	CML	+	No information about radiotherapy
2—3 months	40% mature lymphocytes	A A	+	No information about radiotherapy
3 months	Dominated by blast cells	Blast cell leukaemia	–	No information about radiotherapy
1 month	Hypoplastic with blast cells	Blast cell leukaemia	+	No information about radiotherapy
17 months	?	AML	?	No information about radiotherapy
3½ years	Infiltrated with atypical monocytes and paramyeloblasts	MML	+	No radiotherapy given
4 years	Hypoplastic with 30% myeloblasts	AML	–	No radiotherapy given
6 years	90% lymphoblasts	ALL	?	No radiotherapy given
4 years	Many immature erythroid precursors	AML	+	No radiotherapy given
3 years	Consistent with AML	AML	?	No radiotherapy given
5 years	Dominated by myeloblasts	AML	?	No radiotherapy given
?	?	AML	?	No information about radiotherapy
15 months	Hypocellular >hyperplasia of myelopoiesis	CML	+	No radiotherapy given
2? months	Dominated by myeloblasts	AML	+	No radiotherapy given
12 months	Dominated by lymphoblasts	ALL	+	No radiotherapy given

For explanations see following page

tive streptococcusagglutination reaction Her condition was interpreted as an atypical rheumatoid arthritis In this case it is perhaps difficult to exclude the possibility that the pains originated from the leukaemia although the protracted course makes it appear rather unlikely

Discussion

Since PBZ was first introduced in 1949 many reports describing toxic effects of the drug have appeared Fluid retention, gastro intestinal discomfort (nausea, vomiting, diarrhoea), peptic ulcer, stomatitis, and skin rashes are frequent (18, 19) Some cases of toxic hepatitis have been reported (19) Complications from the haemopoietic system are less frequent but more serious In 1955 Mauer (19) reported 23 fatal complications of PBZ-therapy, twelve of which were due to damage to the haemopoietic system The main manifestations have been leukopenia, agranulocytosis, and thrombocytopenia In addition, aplastic anaemia and isolated cases with depressed erythropoiesis (26) and megaloblastosis (23) have been reported The pertinent literature has been reviewed recently (20)

The mode of action of PBZ on the haemopoietic system is not known Immunologic mechanisms may be operative Hale and de Gruchy (12) thus called attention to the fact that, according to Mauer's (19) review of the toxic effects of PBZ, all cases of agranulocytosis appeared 7—16 days after the beginning of therapy, the toxic effect was not related to the total dose ingested, and nearly all the patients had skin rashes Kjeldsen (16) and Weissmann and Xefteris (30) demonstrated leukoagglutinins in the serum of a patient with PBZ induced leukopenia, while other authors have failed to find this (14) In one case in which anaemia developed during treatment with PBZ, serum from the patient inhibited erythropoiesis in marrow cultures, the inhibitory effect disappeared after steroid therapy (26) In this context it is interesting to note that PBZ is a derivative of amidopyrine In amidopyrine agranulocytosis, serum factors which cause leukocyte agglutination *in vitro* and leukopenia *in vivo* have been unequivocally demonstrated (15 22, 27) The mechanisms which lead to aplastic anaemia may be different Aplastic anaemia developing after administration of PBZ seems to be more dependent on the quantity of the drug ingested, and usually it does not manifest itself until after 4 to 30 months of

TABLE I (Cont) Explanations

Ac arthr	Acute arthritis	Spond	Spondylitis	CML	Chronic myelocytic leukaemia
Arthr	Arthritis	A A	Aplastic anaemia	L S	Lymphosarcoma
O A.	Osteo arthritis	ALL	Lymphoblastic leukaemia	MML	Myelo monocytic leukaemia
R A	Rheumatoid arthritis	AML	Myeloblastic leukaemia	R S	Reticulosarcomatous
Latent period	time from start of PBZ therapy to diagnosis of leukaemia				

treatment Allergic manifestations like the skin rashes associated with PBZ agranulocytosis have not been observed in conjunction with PBZ aplastic anaemia

It is well known what appears to be aplastic anaemia may develop into leukaemia, similarly, both aplastic anaemia and leukaemia may be induced by ionizing radiation and benzene It is an important but undecided question whether aplastic anaemia merely predisposes to leukaemia or whether aplastic anaemia and leukaemia are basically one disease

Since 1958 22 cases of leukaemia associated with the administration of PBZ have been reported (table I) In 19 of these cases the diagnosis appear well established whereas in three cases it is equivocal Thus in the fifth of Bean s (2) six cases two consecutive lymph node biopsies showed a picture consistent with malignant lymphoma or lymphocytic leukaemia but the bone marrow and the leukocytes of the peripheral blood were normal In the same author s third case the bone marrow was infiltrated by anaplastic malignant cells An axillary gland showed disorganization of the normal structure by anaplastic cells A renal biopsy showed the organ infiltrated with similar cells The peripheral blood revealed an increasing number of large immature cells considered to be either lymphocytes or monocytes These findings could be due to leukaemia malignant lymphoma or a generalized solid tumour Post mortem examination was not performed In Garret s (11) case the patient had pancytopenia 96 per cent of the leukocytes in the peripheral blood were lymphocytes, many of which had nucleoli and little cytoplasm Autopsy showed a mainly hypocellular marrow with islands of cells, mostly of the lymphatic series Liver and spleen showed no cell infiltration It may have been a case of aplastic anaemia, as the presence of some immature lymphocytes in the blood is not rare in this condition

Thorpe s (28) patient developed a painful swollen ankle 4—5 weeks before the leukaemia was detected It is possible that the arthritis in this case was an initial symptom of the leukaemia

Fourteen of the nineteen verified cases of leukaemia were acute leukaemia (eight myeloblastic, three lymphoblastic, one myelomonocytic, and two blast cell leukaemia) The remaining five cases were chronic myelocytic leukaemia The high incidence of myeloblastic leukaemia is probably due to the age distribution of the patients, myeloblastic leukaemia being predominant in adults It is interesting to note that no reports associating lymphocytic leukaemia with PBZ have been published

In all cases but one, the indication for treatment had been long standing symptoms from the locomotor system particularly arthritis and spondylitis

In ten of the nineteen bona fide cases reported no radiotherapy had been given In the remaining nine cases no information is available

The interval from beginning of exposure to the development of leukaemia (latent period) has for well known leukaemogenic agents such as ionizing radiation and benzene been shown to vary from two to ten years (8, 10 25)

and three to twenty five years (3, 9, 21, 29) respectively In the nineteen reported cases of leukaemia in patients treated with PBZ (our own cases included), the interval from start of therapy to diagnosis varied from four weeks to six and a-half years In nine cases the latent period exceeded two years Applying the lower limit of the latent period in radiation leukaemia (two years), a causal relationship between the ingestion of PBZ and the development of leukaemia seems unlikely in eight of the cases recorded

The duration of the PBZ therapy and the total dose given vary considerably In general, patients with a latent interval exceeding two years had received a larger total dose of PBZ than patients with a shorter latent period This is to be expected in retrospective surveys of drug exposure

While the previous reported cases of leukaemia associated with PBZ ingestion had been isolated reports, Woodliff and Dougan (31) examined a material of patients with acute leukaemia and found that eight out of fifty five patients had a history of PBZ medication In two of the cases radiotherapy had also been given, and in a third case PBZ was not administered until shortly before the leukaemia became manifest If these three cases are omitted the incidence of PBZ ingestion among their patients with acute leukaemia was nine per cent The authors have compared this material to a group of patients with chronic leukaemia, lymphoma, and 'allied disorders", and found an incidence of PBZ ingestion of 1 2 per cent It is doubtful, however, whether these two materials really are comparable Thus, no information was given of the age distribution of the two groups Since PBZ is often prescribed for "rheumatoid" complaints in elderly people, and acute leukaemia occurs particularly frequently at the extremes of life, a spurious association between acute leukaemia and PBZ may be obtained unless a control group is carefully matched with respect to age

In our material three of the fifty patients had a history of previous PBZ ingestion, i e an incidence of six per cent However, the latent interval is less than two years in two of the three patients On the other hand, since no patient was questioned with special reference to previous PBZ therapy this obviously is a minimum figure

In discussion of the possible leukaemogenic effect of PBZ it must also be considered that an increased incidence of all types of leukaemia, lymphosarcoma, and Hodgkin's disease among patients with rheumatoid diseases have been reported (1, 17) Unfortunately the disorders included under this heading have not been specified

From the available evidence it must be concluded that a possible association between PBZ ingestion and leukaemia has not been proved Prospective studies of patients with well defined rheumatoid diseases receiving PBZ and of similar patients without PBZ medication are clearly needed

Summary

A retrospective survey of fifty patients with acute leukaemia with respect to phenylbutazone exposure is reported

An incidence of six per cent was found The pertinent literature has been reviewed, and the question of the possible leukaemogenic action of phenylbutazone is discussed It is concluded that an association between phenylbutazone ingestion and development of leukaemia cannot be proved from the *available* evidence

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Post-partum Congestive Heart Failure

Beri beri Heart Disease

By

SIGRID DAAE BLEGEN[1]

Material

During 5 months from early October 1963 to March 1964 28 females were admitted to the department because of a rather abrupt onset of severe dyspnea palpitation and edema in close connection with a normal delivery The pregnancies had been without known complications except for in some instances the occurrence of slight signs of toxemia during the later stage Most of the patients were admitted as emergency cases because of severe respiratory and cardiac distress In all cases symptoms and signs of thiamine deficiency was found In 27 instances the case history revealed prolonged malnutrition because of extreme poverty Seven cases were later excluded from the material Other organic diseases might have been the main or an additional cause of the condition These were two cases of far advanced pulmonary tuberculosis 2 cases of mitral valvular disease diagnosed years before 1 case of liver cirrhosis and 1 case of cancer uteri and mitral valvular disease The remaining 21 cases fulfilled the criteria for a diagnosis of beri beri heart disease All patients were examined and controlled clinically by the author

Diagnostic criteria

1 Sudden onset of severe signs of heart failure with edema in close connection with delivery and lactation in individuals without previous history of heart disease
2 Positive history of prolonged malnutrition.
3 Heart enlargement (clinically and by X ray) with marked decrease in size after treatment with thiamine or post mortem cardiac findings compatible with beri beri
4 Eletrocardiographic changes consisting mainly of ST T flattenings and inversions
5 Dependent edema perhaps with ascites or pleural effusion
6 Normal blood pressure or normalization after a few days in hospital
7 Definite signs of polyneuritis i e pares tesias sensibility and (or) reflex changes and (or) other signs of avitaminosis such as skin changes cheilosis and glossitis

Results

Findings

The most important findings are presented in the following figures and tables

Table I shows the time from the delivery to the onset of the severe heart symptoms in the 21 cases It

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Fig 1 A Patient No 2 January 15th 1964

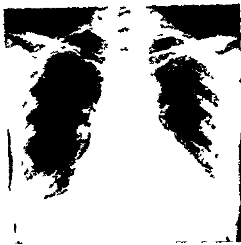


Fig 1 B Patient No 2 January 31st 1964

Patient No 16 died 3 1/2 hours after admission An autopsy revealed a hyper trophic and dilated heart and extensive mural thrombosis in both ventricles The changes in the heart muscle were compatible with beri beri according to the report from the pathology department

A positive history of long standing malnutrition because of extreme poverty was found in all but one of the 21 cases In this case where the economy was not so bad the patient had suffered from marked anorexia throughout the pregnancy and had therefore subsisted on a very insufficient diet

In 14 cases the blood pressure was elevated on admission but became normal after a short stay in hospital

Only in 1 case were the ECG findings normal on admission The ECG findings on discharge are shown in table III

Transient auricular fibrillation was seen in only 1 or possibly 2 cases The most frequent finding from auscultation of the heart on admission was gallop rhythm and a systolic murmur at apex grade II to III In 9 cases auscultation was completely normal on discharge, and in the rest only a soft systolic murmur remained

As shown in the table definite signs and symptoms of polyneuritis were found in all but one of the 21 patients This case had a marked loss of hair In addition to what the table shows, glossitis was found in 1 case and in 2 cases increased deep muscular tenderness Two of the patients were extremely ill at the time which made the evaluation of sensibility changes very difficult In these instances (No 9 and No 16) there may very well have been parestesias and sensibility loss in addition to the findings reported

As shown in the table the occurrence of cheilosis keratotic skin, parestesias, sensibility and reflex changes were

TABLE I The time from the delivery to the onset of the severe heart symptoms in the 21 cases. Cardiac findings on admission and after therapy, symptoms and signs of polyneuritis and avitaminosis

Patient no	Time from delivery to onset of heart symptoms (days)	Edema on admission	Decreased roentgenological heart size after therapy	Symptoms or signs of polyneuritis and avitaminosis				
				Ch	K	P	R	S
1	7	No	Yes	×	×	×		×
2	10	Yes	Yes					×
3	> 60	Yes	Yes	×			×	
4	20	Yes	Yes	×		×		×
5	< 1	Yes	Yes		×	×		×
6	< 1	Yes	Yes					×
7	30	Yes	Yes		×	×		×
8	1	Yes	Yes	×	×	×		×
9	30	Yes	No		×		×	
10	< 1	No	No control	×	×	×	×	
11	7	No	Yes	×	×	×		×
12	60	Yes	Yes	×	×	×		×
13	2	Yes	Yes					
14	30	No	Yes				×	×
15	1	Yes	Yes		×			×
16	< 1	Yes	No	×	×		×	
17	3	Yes	Yes	×		×	×	×
18	2	Yes	Yes	×	×	×	×	×
19	8	No	Yes	×			×	×
20	1	Yes	Yes					×
21	7	Yes	No	×	×		×	×

Abbreviations Ch cheilosis K keratotic skin P parestesias, R reflex changes S sensibility changes

also shows cardiac findings on admission and after therapy, and symptoms and signs of polyneuritis and avitaminosis

In addition to what the table shows there can be added None of the patients had a history of previous heart disease Enlarged heart was found in all cases on admission (clinically and by X ray) The size was in most cases markedly diminished after a few days in hospital as seen in the table

Only in 2 cases did we observe no decrease One of these patients died after 7 days in the ward (No 9) An autopsy revealed an enlarged and dilated heart and extensive mural thrombosis in both left and right ventricle, with embolism to both coronary arteries resulting in cardiac infarction

The other patient (No 21) died suddenly in her home one month after discharge

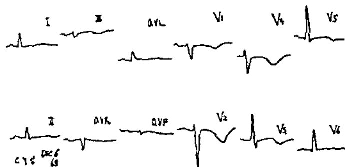


Fig 3 A Patient No 7 December 6th 1963

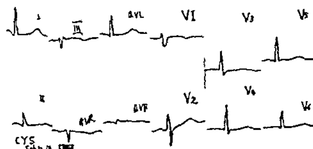


Fig 3 B Patient No 7 February 12th 1964

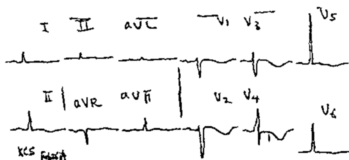


Fig 4 A Patient No 18 February 26th 1964

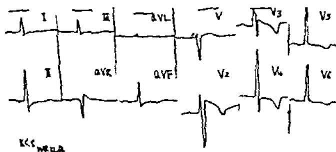


Fig 4 B Patient No 18 March 12th 1964



Fig 2 A Patient No 11 November 27th, 1963

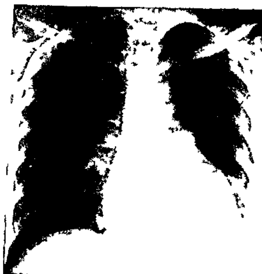


Fig 2 B Patient No 11 December 6th, 1963



Fig 2 C Patient No 11 December 12th, 1963

common findings, and often present in a marked degree The skin changes and paresthesias disappeared after some weeks in hospital, but in several cases some sensibility loss and reflex changes remained, although always less marked

Only 5 cases had no edema on admission Some had received irregular diuretic treatment beforehand

The table shows how the time from delivery until onset of severe heart symptoms varied considerably from a few hours to more than two months

The figs 1 and 2 demonstrate typical heart X-rays selected from 2 of the cases The first film was taken as soon as possible after admission, and the second 2 weeks later (Case No 2) In case No 11 a third film was taken a further week later

The figs 3 and 4 demonstrate the typical electrocardiographic changes in 2 patients (No 7 and No 18) In the first case ECG was normalized 1 month after discharge In the second case there came a development compatible with cardiac infarction The differential diagnosis between the usual beri beri changes in ECG and cardiac infarction was sometimes hardly possible

Table II shows the age distribution (average 30 2 years), number of pregnancies (average 3 7), duration of severe manifest heart symptoms on admission (from 3 days to 2 years), and general

TABLE III The relationship between the clinical condition on discharge and the electrocardiographic and roentgenologic findings

Clinical condition	Electrocardiographic findings on discharge			Roentgenological heart size on discharge			
	Normal	Pathological	Not controlled	Normal	Reduction still enlarged	No reduction	Not controlled
Very good	4	5	1	5	4		1
Good	1	6		3	3	1	
Poor		2		1	1		

TABLE IV The number and localization of arterial emboli

Patient no	Cerebral embolism	Coronary embolism	Pulmonary embolism	Embolism to other organs	Total number of emboli
3	[3]1	[2]1			2
4	1				1
7			[4]1		1
8			[4]1		1
9		[1]2	[1]1	[1]2	5
13			[4]1		1
16	1				1
17			1		1
18	2	1			3
19	[3]1				1
21	[4]1				1
Total number 11	7	4	5	2	18

Diagnosis based on [1] Autopsy
ECG changes and clinical condition
Clinical manifestation revealing sequelae after cerebral lesion
Case history

3 times during 5 months the last time with ECG changes and clinical conditions as in acute cardiac infarction

Table III shows the relationship between the clinical condition on discharge and the electrocardiographic and roentgenologic findings

In 5 cases where ECG had become normal the heart size was also normalized If the heart size did not become normal, the same held for the electrocardiograms

Table IV shows the number and localization of arterial emboli The

TABLE II The age distribution, number of pregnancies, duration of severe manifest heart symptoms on admission, and general clinical condition on discharge and on later control

Patient no	Age (years)	No of pregnancies	Duration of heart symptoms on admission (months)	General condition	
				On discharge	At later check ups
1	39	5	<1	Very good	Good
2	25	3	3	Good	Poor
3	30	1	11	Poor	Readmitted twice
4	28	5	12	Good	Readmitted once
5	36	6	24	Good	No control
6	43	8	2	Very good	Good
7	31	5	2	Good	Very good
8	31	3	3	Very good	No control
9	25	1	6	Died in hospital	
10	42	7	6	Good	No control
11	32	4	2	Very good	Very good
12	25	2	7	Very good	Very good
13	27	4	1	Very good	Very good
14	31	2	5	Very good	Very good
15	30	3	12	Very good	No control
16	23	3	9	Died in hospital	
17	21	2	12	Good	Good
18	33	5	<1	Poor (complete invalid)	No control
19	37	7	<1	Very good	No control
20	22	1	1	Very good	No control
21	24	1	5	Good	Died suddenly at home after 1 month

clinical condition on discharge and on later control Among these 21 cases, 13 had some slight edema during the last trimester of the pregnancy The duration of the hospital stay among the 19 patients varied from 14 days to 109 days with an average of 34 days One of these patients was readmitted twice and another once

The term "very good" means that the patient was free from clinical symptoms and signs of cardiac failure In some instances there were still some peripheral sensibility changes and enlargement of the heart "Good" means that there still might be slight dyspnea on exertion and in some cases slightly enlarged liver and (or) heart (in 1 case no reduction of heart size) Among the "poor", 1 patient was hemiplegic and aphasic because of cerebral embolism (No 18) One other was hemianoptic from the same cause This patient (No 3) suffered recurrent attacks of heart failure with increased and later decreased heart size She was admitted

TABLE III The relationship between the clinical condition on discharge and the electrocardiographic and roentgenologic findings

Clinical condition	Electrocardiographic findings on discharge			Roentgenological heart size on discharge			
	Normal	Patho-logical	Not con trolled	Normal	Reduction, still enlarged	No re duction	Not con trolled
Very good	4	5	1	5	4		1
Good	1	6		3	3	1	
Poor		2		1	1		

TABLE IV The number and localization of arterial emboli

Patient no	Cerebral embolism	Coronary embolism	Pulmonary embolism	Embolism to other organs	Total number of emboli
3	*1	*1			2
4	*1				1
7			*1		1
8			*1		1
9		2	*1	*2	5
13			*1		1
16	1				1
17			*1		1
18	*2	1			3
19	*1				1
21	*1				1
Total number 11	7	4	5	2	18

Diagnosis based on * Autopsy
* ECG changes and clinical condition
Clinical manifestat on revealing sequelae after cerebral lesion
* Case history

3 times during 5 months, the last time with ECG changes and clinical conditions as in acute cardiac infarction

Table III shows the relationship between the clinical condition on discharge and the electrocardiographic and roentgenologic findings

In 5 cases where ECG had become normal the heart size was also normalized If the heart size did not become normal, the same held for the electrocardiograms

Table IV shows the number and localization of arterial emboli The

diagnosis was made either by the past history or by sequelae after cerebral embolism, or by ECG changes and a clinical condition compatible with cardiac infarction or at autopsy

Attention is called to the fact that peripheral thrombophlebitis was never observed in spite of a rapid dehydration in most cases Nineteen patients lost on the average 9 2 kg during the first week The highest value was 22 7 kg One of the patients lost 7 9 kg during the first 24 hours

Pulmonary embolism was supposedly caused by mural thrombi in the right heart In 11 of the 21 patients symptoms and signs of embolism were found Three cases showed evidence of both cerebral and coronary embolism In 1 of these cases (No 9) embolism to the lung and liver and spleen was also found at autopsy The total number of diagnosed emboli in the 11 cases were 18

Treatment

The treatment consisted in bed rest low-salt diet, digitalization, diuretics and intravenous thiamine, 100 to 200 mg per day In addition B-vitamins was given by mouth

Because of the striking tendency to arterial embolism, some cases were also given dicumarol, the treatment being controlled by Owren's Thrombotest method

Discussion

Since early in 1958 Scandinavian doctors at National Medical Center in Korea have observed cases of congestive heart failure in connection with pregnancy, delivery and lactation (8)

Many of these patients had no previous history of heart disease or toxemia That B_1-avitaminosis was the main cause had been discussed, but there was never any common agreement The cases were classified as post partum congestive heart failure Sometimes they were named beri beri hearts, but the diagnosis was not proved One reason was that determination of vitamin B_1 in blood or urine was out of question for technical reasons Pyruvic acid determination was tried, but the laboratory results were found to be unreliable

Systematic neurological examinations had not been done previously, perhaps because of the old classification of beri beri into 3 clinical types, i e a) the wet type, with edema and serious effusion, b) the dry type, with affection of the peripheral nervous system, and c) the fulminant type characterized by acute cardiac symptoms Four cases have previously been reported from the hospital with autopsy findings described as typical for beri beri heart disease although not pathognostic (5)

Beri-beri has been considered mainly an oriental disease, among populations where the main food consists of rice or other cereals More recent studies have shown that the disease occurs also among western people but usually in a milder form (11, 12) In U S A studies have shown a rather high percentage of beri beri among alcoholics and mental patients

In 1945 Blankenhorn suggested rather simple criteria for the diagnosis (2, 3) She called attention to a more chronic type of heart failure due to B_1 avita-

minosis, different from the fulminant type Her criteria have been used in this study (see above) Blankenhorn made it clear that there is no real difference between the 3 types' Several manifestations may be present, but with one or two signs predominating, changing perhaps from time to time

Complete recovery may take place if treatment is started in the early stage but recovery may be incomplete if the myocardium has been damaged by more advanced degenerative changes (1) Other investigators claim that the heart disease is due to reversible biochemical abnormalities not characterized by necrosis, fibrosis or other irreversible processes (6 9)

With regard to the conditions that may lead to vitamin B_1 avitaminosis only scanty information is available about Korean food But from what little may be found it appears that the intake of vitamin B_1 and also other B-vitamins is low in Korea Pellagra is quite common (13)

Besides there is the extremely one sided carbohydrate diet which increases the demand for vitamin B_1 When the extra demand from pregnancy delivery and lactation in combination with poverty is added it would not be surprising if cases of beri beri were to occur

Our observations point to a very large number of such cases Our material was obtained during a period of only 5 months After that time we saw a considerable number of similar cases and we have reasons to believe that the condition described is frequent in the lower social strata of the Korean population

In our experience the prognosis was not bad, if treatment was started before cardiac decompensation had gone too far or arterial embolisms had done too much damage

In 7 females who had been living alone during the larger part of the pregnancy without financial help (husbands were dead or had left), the emaciation was extreme and the hearts were much enlarged Nevertheless 4 of them regained normal heart size in hospital The duration of cardiac symptoms in these cases varied from one week to 7 months before admission

Most of the patients returned to heavy manual work after discharge Those who came regularly for ambulatory control after discharge were given multi vitamin B tablets (Multiplex) in addition to other necessary treatment such as digitalis and diuretics

The main causative factors were 1) poverty with a diet consisting almost exclusively of cereals and cheap vegetables 2) hard manual work 3) pregnancy delivery and lactation There was at the time no possibility for more extensive research In the future this should be directed towards following problems

1) A biochemical proof of B_1 avitaminosis by vitamin B_1 determinations or, more simply by pyruvic acid determination in blood before and after treatment with thiamine and with glucose loading, or other suitable tests (4, 7)

In May 1964 a new effort was made to determine pyruvic acid in the blood of patients with beri beri heart disease The laboratory started with the enzymatic method described by Vincent

Clarks in 1961 When this was written it was too early to tell if the method was working satisfactorily

2) A search should be made for the cause of the intracardial thrombosis, whether it is due to hypercoagulability of the blood or to other factors In this connection it is again stressed that we never saw peripheral thrombophlebitis in spite of the rapid dehydration which took place during the first days in the hospital

The possibility exists that cardiac ischemia may play a role in the mural thrombosis On the other hand, the reversibility of the heart condition speaks against a severe ischemia

3) It would be of the greatest interest to have some of these patients followed over a prolonged period What is the end result of extensive mural thrombosis? Organization with endocardial fibrosis?

During a whole year only 1 case of beri beri was seen in a male He had been living on an unusually strict rice-carbohydrate diet prescribed by a 'herb-doctor"

Summary

Although the final biochemical proof of the diagnosis beri beri is lacking in these 21 women with cardiac and peripheral nerve disease, the findings are so uniform and convincing that there can be little doubt Beri-beri seems to be a common disease in Korean women living under very poor economic conditions The disease is especially apt to occur in connection with pregnancy, delivery and lactation The symptoms may last for months and even years, or may be of more acute character, sometimes leading to death In this material death or lasting damage was due to arterial embolism, as a rule caused by mural thrombosis in right and left heart

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Insulin-Glucose-Potassium Infusion in Acute Myocardial Infarction

By

T Lundman and E Orinius

Sodi Pallares et al (6) in 1962 presented their preliminary results of treatment with insulin glucose potassium infusion in acute myocardial infarction The infusion was usually given every other day as a 1 000 cc 5—10 % glucose solution with 20 IE insulin and 40mE potassium chloride During the treatment the upward shift of the S T segments and the frequency of arrhythmias seemed to decrease and afterwards to increase again at the end of the infusion However these authors had no controls

The theory of this treatment was briefly that the damaged myocardial cells would be kept polarized until a collateral circulation had developed The transmembrane potential largely depends on the intra extracellular potassium gradient As the intracellular potassium concentration increases when glucose enters the cells the insulin would be the most important part of the therapy

The hospital mortality in acute myocardial infarction is 30—40 % about half of it being caused by arrhythmias which are most frequent during the first days (1, 9) For the rest the major cause of death is shock, which is considered as a contraindication to potassium infusion because of renal impairment A decrease in arrhythmia mortality could thus be the greatest short time result of the therapy

As an antiarrhythmic the therapy should be started as soon as possible and be given continuously The insulin dose should — as the active principal of the therapy — be as great as possible

Modified after these principles the insulin glucose potassium treatment in acute myocardial infarction was given as a controlled clinical trial in our department in 1962 The only conclusive result would be a mortality difference between the treated and the control group which would require large series A short cut to a negative result in regard to decreased mortality from arrhythmias would be to study the frequency of all arrhythmias (fatal or not) in small series by frequent ECG recordings, since

Submitted for publication May 5 1965

TABLE I Treated cases

Sex	Age	GOT max	ECG recordings with arrhythmias, %		
			Day 1	Day 2	Day 3
♂	72	150	0	6	0
♂	57	367	37	8	6
♂	40	290	0	0	0
♂	41	105	0	0	0
♂	41	228	0	0	0
♂	43	175	39	0	0
♀	62	70	0	0	0
♂	66	265	0	9	0
♂	73	170	0	0	0
♂	65	148	4	2	0
♀	59	106	0	0	0
♂	68	234	0	0	19
♂	72	238	7	0	0
n=13	m=58	m=196	m=7	m=2	m=2

arrhythmias on the whole mean a raised mortality risk in acute myocardial infarction (2, 4, 7, 8)

The results with regard to the effect of the therapy on the frequency of all arrhythmias are now available

Material

Of the patients with acute myocardial infarction in our department those were selected who a) were diagnosed within 24 hours of the onset of the chest pain b) did not have diabetes mellitus, and c) were not having digitalis therapy The material consists of 24 men, 40—76 years old and 2 women 59 and 62 years old In another case the therapy was started but had to be stopped after 12 hours owing to blood pressure fall There was no significant difference between the groups with regard to age (mean = 58 and 60 years respectively) or glutamic oxalacetic transaminase maximum (mean = 196 and 226 modified Karmen Wroblewski units/cc respectively normal 10—35 units /cc)

Method

All 26 patients got the usual infarction therapy with absolute bed rest and sedatives, usually barbiturates and dicumarol as anti coagulant In addition every other patient (selected by lot) had continuous infusion therapy from the time of diagnosis until 72 hours after the onset of chest pain receiving 60 IE standard insulin and 45 mE potassium chloride in 1 500 cc 10 % glucose every 24 hours During the same 72 hours the ECG was recorded in all patients every 30 minutes for 30 seconds with a paper speed of 25 mm/sec $V_{1,2}$ or CR_1 and sometimes other leads were recorded At the ECG examination the number of recordings with any arrhythmias was given as the percentage of the total number of recordings per 24 hours Premature beats were noted as arrhythmias if they appeared in a frequency of 1/10 or more

Results

The single complication of the therapy was thrombophlebitis in the infusion

TABLE II Control cases

Sex	Age	GOT max.	ECG recordings with arrhythmias %		
			Day 1	Day 2	Day 3
♂	75	28	80	21	33
♂	53	79	20	0	0
♂	64	220	24	0	0
♂	53	380	10	6	0
♂	66	96	0	0	3
♂	76	180	3	75	14
♂[1]	71	440	0	0	0
♂	57	260	0	41	0
♂	54	82	0	0	0
♂	59	290	0	0	0
	67	189	12	17	46
♂	41	435	0	0	0
♂	50	225	0	0	0
n 13	m 60	m−226	m≈12	m −12	m 7

[1] Died on the 9th day

vein in some cases which might have been due to the polyethylene catheter used in the beginning of the investigation When we had changed to infusion through a needle no thrombophlebitis was noted

No cardiac asthma or pulmonary oedema occurred nor any symptoms of hypoglycaemia Routine determinations of blood glucose and serum potassium every morning during treatment showed normal values There was no shock complication (except the preshock state in the excluded case) One death occurred in the control group, none in the treated one This patient had no arrhythmia recorded during the three control days but on the 4th day developed an auricular flutter which persisted until sudden death on the 9th day The autopsy revealed advanced coronary sclerosis and a myocardial infarction of the left ventricular wall The infarction was about 10 days old

It appears *from the tables* I *and* II that the frequency of ECG recordings with arrhythmias is lower in the therapy group than in the control group for all three days and the difference is probably significant when comparing the means of the treatment and control groups for day 1+2+3 ($0.05 > p > 0.01$) as well as for day 2+3 ($0.05 > p > 0.01$) Day I may be excluded in the comparison since the effect of the treatment probably has some delay, but this does not alter the significance of the difference The arrhythmias have mostly been premature beats, but also periods of sinus arrhythmia auricular fibrillation, and AV block I have been recorded

Discussion

After the start of this investigation Sodi-Pallares et al (3) have published a controlled clinical trial in 25 cases with 25 controls, where most of the patients got a continuous infusion of 10 % glucose added with 25 IU insulin and 40 mE potassium chloride during the first 72 hours In their investigation the arrhythmias were measured by its duration, which was 4 days for the treated group and 11 for the control group Though this treatment was not continuous in all cases and the insulin dose was small, their results point in the same direction as ours The results of these investigations together give cause for another clinical trial of the therapy with mortality as the principal measure

Opposed to this is the fact that Johansson et al (5) did not find any significant difference in mortality in a material of 108 cases, half of which were controls, but they gave the treatment with glucose-insulin-potassium only for some hours every day and the results are consequently not comparable

We have avoided the use of this therapy in patients with shock or preshock due to the possibility of oliguria occurring in them The hazard of a tendency to induce hyperkalaemia in such a situation must be balanced against the possible benefits of the therapy Until the effects of such an infusion are more completely understood, it should probably be omitted in the treatment of such patients

Summary

By treatment with a continuous infusion of insulin glucose potassium in acute myocardial infarction until 72 hours after its onset, the frequency of all arrhythmias was reduced in a probably significant way, in comparison with a simultaneous control group ($0.05 > p > 0.01$) This result gives cause for further clinical trials with mortality as the principal measure

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Renal Hypertension Caused by Retroperitoneal Hematoma

By

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In addition to the usual causes of renal artery stenosis, i e, atherosclerosis and fibromuscular hyperplasia, other more rare etiological factors have been infrequently recorded Among this spectrum are ganglionneuroma (6), aberrant vessels (1, 2) aberrant muscle bundles (3 8), and periarteritis nodosa (2 8)

Direct renal trauma has usually been connected with the subsequent hypertension by two mechanisms either by the degenerative changes following parenchymal damage or by the compressive effect of a subcapsular hematoma on the intrarenal vasculature The second route of action has been regarded as the clinical image of the cellophane nephritis of Page (4) Case records of both types of renal hypertension have been published since 1940 (5, 9, 10 13) The present report describes a case of renal artery stenosis evoked by retroperitoneal hematoma

Case report

The patient was a 47 year-old technician with a non-contributory medical history in childhood and during adolescence In 1938 he fell from a telephone pole and received a strong blow on the right side of the body resulting in multiple costal fractures and a hepatic rupture He was treated without operative intervention in a municipal hospital with full recovery He was in combat service during the Second World War suffering occasionally from abdominal discomfort In 1957 he was treated in a municipal hospital because of a duodenal ulcer

Since 1961 he had experienced effort dyspnea and the blood pressure was observed to be elevated He was digitalized and an antihypertensive treatment was started with reserpine The dyspnea of effort was relieved but the blood pressure continued to be high Following a period of treatment with thiazide diuretics the blood pressure gradually fell Since 1962 he was distressed by abdominal pain not connected with food intake In August 1963 there was an onset of sudden pain in the right upper quadrant of the abdomen, persisting for 10 hours Similar episodes recurred and the patient was admitted to a local hospital The right subcostal area was very tender on pressure as also was the back at the level of the right kidney B P was 260/160 A hard nodular tumor was palpated on the right side of the abdomen The case was at first treated as renal colic, but when intravenous pyelography aroused a suspicion of tumor in the right kidney the patient was transmitted to the First Medical Clinic in Helsinki

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Fig 1 The renal angiogram showing an occlusive lesion in the proximal segment of the right renal artery

On admission the patient was pale and in a poor general condition He complained of abdominal distress and of dyspnea even at rest B P was 210/140 The eye grounds were of class III (K-W) There was a protodiastolic gallop rhythm but no murmurs The femoral arteries were normal on palpation Chest X ray showed slight venous congestion of the lungs and a heart of 850 cc/m² BSA with a prominent left ventricle ECG revealed hypertrophy and systolic overloading of the left ventricle The urinary sediment was normal Creatinine was 1 52 The acid base balance was normal as also were the serum electrolytes There was no anemia leukocytes were 10 800 per mm³ with a shift to the left Normal values were found for the daily excretions of catecholamines and vanillin mandelic acid as well as of the urinary 17 ketosteroids and 17 OH-corticoids and for their response to ACTH

Because of the palpable abdominal tumor the intravenous pyelography was repeated The left kidney was normal The upper calices of the right kidney were depressed caudally and a pathological density was located around the upper pole The finding was suggestive of either a hypernephroma or an adrenal tumor A transfemoral renal angiography was performed to confirm the diagnosis The left kidney was supplied with two renal arteries and was normal About one cm from the beginning of the right renal artery there was a classical stenosis with post stenotic dilatation (fig 1) The branches appeared normal but were displaced caudally The middle suprarenal artery was also filled with contrast Two branches of this artery about 5 cm apart surrounded the tumor like shadow in the upper pole of the kidney The pathological density was about 6 × 9 cm in area No area of increased vascularization was observed

After a short period of antihypertensive treatment resulting in a B P of 195/115 the patient was sent for operation The right kidney was exposed through a Nagamatsu incision and a large hard tumor extending from below the diaphragm to the pelvis was found It was closely adherent to the adjacent tissues and completely surrounded the kidney, which could not be palpated separately The tumor was dissected free with difficulty because it was adherent to the inferior vena cava and to the descending part of the duodenum The renal artery was dissected free and ligated The arterial wall showed arteriosclerotic thickening on palpation The removed preparation was about the size of three fists It consisted of hard outer layer about one inch thick covering a loose hemorrhagic mass and cavities filled with fluid Macroscopically the kidney and the adrenal were normal The surgeon's tentative diagnosis was a retroperitoneal sarcoma

Microscopically the specimens of the adrenal a lymph node and the renal artery were normal Only slight degenerative changes were observed in the glomeruli and renal arterioles The specimen of the tumor mass showed no malignant features but was composed of granulation tissue including hemosiderin rich macrophages and a capillary network The finding was typical of an organized hematoma After this discovery the whole removed specimen was microscopically examined with essentially the same findings

The blood pressure response to the nephrectomy was a decline to 150/95 after six days The eyeground regressed to grade II and the heart volume declined by 200 cc/m² BSA

The ECG signs of left ventricular systolic overloading diminished. The patient was discharged in good condition in December 1963 one month after the operation with a B P of 180/90 and no antihypertensive medication. His blood pressure was controlled by his private physician. It slowly rose to 220/120 in April 1964 when an antihypertensive treatment was begun with good response. He was readmitted to the clinic in December 1964 for control studies. On admission he was on a combined reserpine thiazide regimen. The blood pressure was 150/90 with a rather wide fluctuation during the hospital stay. The eye grounds were of grade II and the ECG and chest X ray findings were unchanged. Creatinine was 1 30—1 64 mg%, and the endogenous creatinine clearance 86 4 ml/min. The patient was discharged with a combined reserpine methyldopa and thiazide medication.

Discussion

In considering the etiology of hypertension in the present case it is obvious that the minimal changes in the glomerules and intrarenal vessels were not commensurate with the level of the blood pressure. Necrotizing arteriolitis, chronic nephritis, tubular atrophy and interstitial fibrosis have been the changes encountered in kidneys being compressed by subcapsular hematomas (4, 10). The renal pathology in this case rather is in favor of a protective action of the stenosis on the intrarenal vasculature. The stenosis itself is most readily explained by the compressive action of the organized hematoma on the renal artery which had to be dissected free from the surrounding mass. The renal artery was not meticulously examined. It gave an impression of arteriosclerotic thickening on palpation but the specimens examined microscopically were normal. Farrel and Young (5) have described a patient analogous in many respects. In their case the kidney was compressed by a *large hemorrhagic cyst* and supplied by two renal arteries exhibiting hyperplastic sclerosis. The renal parenchyma showed, however, gross pathological changes in contrast to the present case.

Hypertension seems not to be a frequent sequela of renal injuries. Sargent and Marquardt (12) reported a series of 200 cases of renal trauma. Follow up showed one case of hypertension which was cured by nephrectomy. Jones (7) reported 24 cases of renal rupture with no subsequent hypertension. The follow up time in these and other similar series is, however, rather short in view of case reports describing established hypertension due to renal trauma in the past history. Ten years (10), 12 years (5) and 23 years in the present case elapsed between the trauma and the discovery of the hypertension.

The finding that hypertension can arise through a renal artery stenosis due to hematoma calls for an extension to the list of indications for angiography in hypertension. This is in accord with the experience of Olsson and Lunderquist (11) who have shown the superiority of renal angiography in renal traumatology in general.

Summary

A 47 year old male patient is described who became hypertensive 23 years after a trauma resulting in hepatic rupture. Angiography revealed renal artery stenosis which was found to be evoked by

retriperitoneal hematoma completely surrounding the right kidney and its major vessels Removal of the organized hematoma including the kidney resulted in regression of the hypertension Later on the blood pressure rose again, but was easily controlled by antihypertensive medication, in contrast to the situation before the nephrectomy

It is suggested that antecedent flank trauma should be included in the indications for renal angiography in hypertensive patients

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Ethanol and the Human Liver

Correlation between Mitochondrial Size and Degree of Ethanol Abuse

By

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Animal experiments have led us to assume that prolonged ethanol consumption causes liver mitochondrial swelling and malformations of such a kind that many functions dependent on an intact structural organization are disturbed (1—5)

The results of these animal experiments are taken as the foundation of our investigations into the consequences of prolonged ethanol consumption on the tissues of human alcoholics This paper deals with the ratio between the number of enlarged liver mitochondria, determined by means of electron microscopy and the degree of ethanol abuse

Material

Liver biopsies were taken with a Vim Silbermann needle as described earlier (6) The liver pieces were immediately chilled in ice-cold isotonic NaCl solution and cut into pieces for electron microscopy as previously described (4) On the electron microscopic pictures the longest axes of at least 500 mitochondria from each patient were measured The objects were picked out at random from various levels of at least two different liver samples from each patient

This investigation includes 39 male patients With a few exceptions (the controls) they had consumed ethanol for several years and were thoroughly questioned about their alcohol consumption as far back in time as they could remember

From the information given which extended over at least the last year we calculated the mean consumption of ethanol irrespective of whether the alcoholic beverage was beer wine or spirits. *On the basis of the mean* values of ethanol consumption the patients were divided into six groups extending from no ethanol at all to 45 cl or more per day calculated as a 45 % ethanol solution

For long periods these patients had consumed considerably more than the calculated mean consumption indicates Between these periods there have been intervals with no or only a slight alcohol consumption Many exhibited the symptoms of an advanced alcoholic disease including altered tolerance towards alcohol, easily started alcohol hunger and need for a morning drink

The intermittent as distinct from continuous drinking pattern and the lenght and intensity of the periods may be significant in

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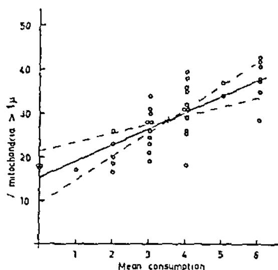


Fig. 1. *Mitochondrial size and the degree of ethanol abuse.* Each dot represents the percentage of mitochondria longer than 1μ (ordinate) in the liver of an individual patient with a mean ethanol consumption given along the abscissa. At least 500 mitochondria from each patient were measured. The patients are distributed in six groups according to their mean consumption where Group 6 represents those with the highest mean consumption (45 cl or more per day). At the zero point on the abscissa are three patients who are teetotallers.
$\bar{x} = 3.51, \bar{y} = 28.6$ (+)
The regression coefficient (b) = 3.78, $t_b = 8.17$, d.f. = 37, $p < 0.001$
The correlation coefficient (r) = 0.803, $t_r = 8.14$, d.f. = 37, $p < 0.001$
$p = 0.001$ will give a population regression coefficient $2.05 < \beta < 5.51$ which is indicated in the diagram by the dotted lines.

the development of subcellular changes. However, as variations in the drinking pattern of this kind are very individual, our restricted material does not at the present stage allow us to take such factors into consideration.

Results

Mitochondrial size and ethanol consumption
From electron microscopic photographs we measured the lenght of the mitochondria in the livers of 39 male alcoholics. On the basis of the mean value of their ethanol consumption the patients were arranged in six groups along the abscissa in fig. 1, with highest ethanol consumption on the right. Along the ordinate is given the percentage of mitochondria longer than 1 μ in the liver of each patient.

A correlation coefficient equal to 0.80 ($p < 0.001$) shows a connection between the degree of ethanol consumption and the mitochondrial size.

Discussion

Human alcoholics may, without any doubt, be regarded as a very inhomogeneous group and with only one factor in common, that is, a more or less pronounced need for ethanol. Differences in age, nutritional state, previous illness (hepatitis, bile obstructions) and abuse of other drugs are some of the variables. Besides, great differences exist as regards the drinking pattern, the amounts of ethanol consumed and the type of spirit used.

Some of these factors are irrelevant to the problem at the present stage and can be eliminated. Thus no patients who had a diagnosed bile obstruction or hepatitis or who abused other substances than ethanol were included. The patients were treated equally as regarded sedatives, vitamins and food after their arrival at the hospital and the biopsies were never taken earlier than one week after arrival.

The patients were not selected by age. However, no correlation could be found between the mitochondrial size and the age of the patients.

In spite of attempts to eliminate as many irrelevant factors as possible the

alcoholics studied still formed a rather inhomogenous group, which meant that exploratory animal experiments, performed under controlled conditions, had to be made The results of these experiments which have been reported elsewhere is summarized as follows A striking consequence of the prolonged ethanol treatment of experimental animals is the high percentage of enlarged liver mitochondria (5, 7) often connected with disorganized cristae (7) the changes partly persisting months after ethanol has been withdrawn (7) The mitochondria from these livers also show a reduced oxidation rate of several substrates (1, 2, 3 5) the ratio between the Mg[2] stimulated and the DNP stimulated ATPases is increased (1) and the content of thiamine diphosphate decreased (8) compared with controls These observations indicate a functional disorder connected with a structural disorganization as a consequence of prolonged ethanol treatment

A previous report (6) shows that in the liver of human alcoholics various types of malformed mitochondria occur This paper shows that the number of enlarged mitochondria is proportional to the degree of ethanol abuse (fig 1)

Thus prolonged ethanol consumption causes deviations of mitochondrial size in man as well as in rats The main types of malformations however differ in the two species (4 6) This makes more doubtful an assumption that the dominant human variant of malformed mitochondria had also lost part of its capacity to perform its normal functions as was the case in the rat This problem is now being investigated

Summary

The size of liver mitochondria from 39 human alcoholics was examined by means of electron microscopy A positive correlation was found between the number of enlarged mitochondria and the degree of ethanol abuse

Acknowledgements

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Book reviews

Atlas of Topographical and Applied Human Anatomy Volume I Head and Neck By Eduard Pernkopf 356 pp, 332 ill W B Saunders Co, Philadelphia and London 1963

This Atlas is incomparably the best book on anatomy I have seen in the course of the years The illustrations are extremely fine, most of them in colour, and they are accompanied by clear and easily read explanatory text The book must be a perfect guide to the student of anatomy As plastic surgeon I recommend it warmly to all engaged in surgical specialties

Karl-Johan Grenabo

Stockholm

Atlas of Topographical and Applied Human Anatomy Volume II Thorax, abdomen and extremities By Eduard Pernkopf 421 pp, 378 ill W B Saunders Co, Philadelphia and London, 1964

The book is an English translation which is issued simultaneously with a new German edition of the well-known Pernkopf's anatomical atlas

The new volume is slightly reduced in size and the illustrations are of rather smaller format, which makes the book easier to handle without impairment of the illustrations The atlas contains 378 illustrations, mostly coloured, of dissected topographical regions As a rule series of dissections are shown at different depths The reference lines from the names in the margin to the anatomical structure are very close together But they are fewer in number than in earlier editions and do not appear to disturb the pictures The illustrations are the same as in earlier editions, which is an advantage They are undoubtedly among the best anatomical prints in existence The nomenclature is fully up-to date

The atlas should be of great value as reference book for surgeons and others working on topographical anatomy

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Takayasu's Disease

Post mortem Examination of a Previously Published Case

By

Kerstin Boström and Ove Hassler

Takayasu's syndrome, pulseless disease and "young female's arteritis" are synonyms of a disease first described by the Japanese ophthalmologist Takayasu in 1908. Compilations of published cases including extensive reports on the various aspects of the disease have been made (1, 3, 10). We found two reasons for reporting the autopsy findings in the following case. Firstly, the clinical picture in this case was carefully described in three publications (1, 3, 9) when the patient was still alive. Secondly, we combined the macroscopical and histological examination with a radiological study of the occurrence of calcifications in the vessels.

Brief clinical history

The clinical history up to 1955 was reported previously (Lindquist's case 2 (9), Ask-Upmark's case (1, 3)). From 1955 to her death in 1964 the patient was treated with cortisone and prednisolone (for the main part Decadron® (Merck Sharp & Dohme) was used in a dose of 1 mg daily). For long periods she was comparatively free from pain and her main complaints were psychic depression and hirsutism. The ESR varied between 5 and 96 mm/hr but was generally about 20 mm/hr. Repeated paper-electrophoresis examinations of the serum proteins gave normal results. Ophthalmological examinations failed to disclose anything pathological. Blood pressure determinations and oscillometric examinations in the arms gave normal results.

From 1959 there were signs of increasing cardiac decompensation. The ECG showed signs of coronary insufficiency and affection of the myocardium. The patient was therefore digitalized. In 1963 X-ray examination of the chest showed a general enlargement of the heart with a volume of 1 160 ml (= 700 ml/m² body surface). On 9 August 1964 she died suddenly at the age of 47 with the clinical picture of left heart failure.

Gross findings at autopsy

GENERAL FINDINGS

A complete post mortem examination was performed 30 hours after death. The body was that of a middle aged woman of ordinary physique. There was no peripheral oedema. The right pleura contained 800 ml of transudate. Apart from fibrosis around the large

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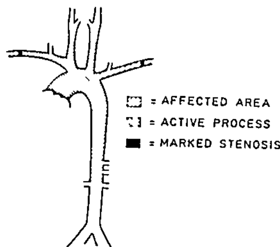


Fig 1 Distribution of affected areas in the aorta and its large branches

vessels, there were otherwise no pathological changes in the mediastinum The lungs showed acute and chronic congestion The liver (weight 2,030 g) and spleen (weight 150 g) were acutely congested The kidneys (each weighed 140 g) showed only slight nephrosclerosis The suprarenal glands (total weight 7 g) were reduced in size but otherwise of normal appearance The other endocrine organs showed no pathological changes In the spinal column there was some osteoporosis The brain (weight 1,320 g) showed a pin sized old softening in the basal ganglia of the left cerebral hemisphere The appearance was otherwise normal The arteries at the base of the brain showed slight arteriosclerosis

CARDIO VASCULAR SYSTEM

Heart The heart (weight 580 g) was moderately dilated The left ventricle was hypertrophied with diffuse slight fibrosis in the myocardium and a slight thickening of the endocardium The coronary arteries showed slight arteriosclerosis

Aorta, large arteries in the neck and proximal parts of the arteries to the arms The aortic wall was changed from its most proximal part to the level of the origin of the renal arteries (fig 1) The ascending aorta and the aortic arch were both wide, the wall here was markedly thickened, leathery and adherent to the surrounding tissues The dilatation and the thickening of the wall decreased distally in the descending and abdominal aorta In the whole intra thoracic part of the aorta the intima was changed In the ascending part the intima was diffusely thickened The aortic arch showed, in addition to a diffuse thickening of the intima, small partly ulcerated, atheromatous plaques which became more numerous in the distal parts In the descending aorta a large number of atheromatous plaques occurred and several of them showed extensive ulcerations. The changes in the intima decreased below the diaphragm, and below the origin of the renal arteries only very slight intimal changes were visible The circumference of the middle part of the ascending aorta was 110 mm, the circumferences of the middle parts of the descending aorta and the abdominal aorta were 60 and 40 mm respectively

The innominate artery, the proximal halves of the left and right common carotid arteries and the subclavian arteries showed a moderate thickening of their walls that was more marked proximally than distally The left common carotid artery showed a slight narrowing of its lumen at its origin, whereas the other vessels had an ordinary width of lumen There was a diffuse, fibrous thickening of the intima and, in addition, solitary small atheromata occurred in the innominate artery At the transition between the axillary and brachial arteries the lumen was almost totally obliterated on both sides The arterial wall was thickened some centimetres further distally The carotid sinus of both sides showed moderate atheromatous changes of the intima

Other vessels The intercostal arteries showed at their origins atheromatous thickening of the intima and in some cases a considerable narrowing of the lumen The renal arteries showed only very slight arteriosclerotic intimal thickening at their origins without narrowing of the lumen Other arteries that departed from affected vessels had only slight atheromatous intimal thickening at their origin Their walls were otherwise of normal appearance Other vessels examined showed only spotty atheromatous intimal thickening

Methods

RADIOLOGICAL EXAMINATION

The aorta the large arteries in the neck, the cerebral axillary brachial radial coronary, pulmonary coeliac (with its large branches) superior mesenteric, inferior mesenteric renal common iliac internal iliac external iliac, femoral and posterior tibial arteries were removed from the cadaver The fixation and X ray examination were performed as reported previously (6)

MICROSCOPICAL EXAMINATION

Specimens for microscopical examination were taken from the formalin fixed vessels and most other organs and were embedded in paraffin The sections were stained with Gomori s aldehyde fuchsin toluidine blue PAS and according to van Gieson Ladewig and von Kossa.



Fig 2 Radiological examination of the aorta The *lumen is opened by a longitudinal incision* and the vessel walls have been folded outwards A. Ascending aorta and aortic arch. The arrow indicates the aluminium foil standard for definition of calcifications. B Descending aorta, showing more and larger calcifications than the parts of the aorta in A C—E Cross-sections through different parts of the aorta. The thickening of the wall is marked in the aortic arch (C) The calcifications of the descending aorta (D) are localized to the intima The abdominal aorta (E) shows an ordinary thickness of the wall Magnification C—E × 3 5

Results of the radiological and microscopical examinations

RADIOLOGICAL EXAMINATION

A total of 3 0 cm² of calcification occurred *in the ascending aorta and the aortic arch* (fig 2 A) In the descending aorta (fig 2 B) and the abdominal aorta proximally and distally to the origin of the renal arteries the areas of calcification were 7 2 cm² 2 0 cm² and 0 2 cm² respectively The calcifications were localized to the intima (fig 2 D) The large arteries in neck had no calcifications apart from the cavernous and petrous portions of right and left internal carotid arteries that had 0 2 cm² of calcification In the splenic artery and in the internal iliac arteries a 0 1-cm² large calcification was found In the other arteries no calcification was discovered

MICROSCOPICAL EXAMINATION

Heart In the sections from the heart hypertrophy of the muscle fibres of the left ventricle and a slight diffuse fibrosis were encountered

Aorta large arteries in the neck and axillary and brachial arteries In these arteries the histological picture was characterized by adventitial and periadventitial fibrosis destruction of the tunica media and changes in the intima

In the adventitia there was marked fibrosis and thickening particularly in the ascending aorta and the aortic arch (fig 3 A) In these parts of the aorta numerous inflammatory cell infiltrates occurred consisting mainly of lymphocytes and plasma cells The infiltrates were often seen in close connection to the *vasa vasorum*, which had thickened sclerosed walls and showed narrowing of the lumina (fig 3 A) In the ascending aorta and proximal half of the aortic arch some *vasa vasorum* showed destruction of the elastic tissue and loss of the normal structure of the wall (fig 3 C) There were scattered inflam-



Fig 3 Aortic arch Intima to the left Adventitia and periadventitial tissue show a *marked thickening and fibrosis* The vasa vasorum have thickened walls Infiltrates of inflammatory cells occur in periadventitial tissue B Ascending aorta The media is to a great extent replaced by granulation tissue containing *multinucleated* giant cells (arrow) (*Intima to the left adventitia to the right*) C Ascending aorta One *vas vasorum* shows destruction of its wall In the surroundings scattered inflammatory cells are seen Gomori s aldehyde fuchsin combined with van Gieson s stain Magnification A × 13 B × 45 C × 85

matory cells in the surrounding tissues No fibrinoid necrosis could be discovered The inflammatory cell reaction was sparse in the innominate artery and at the origins of the common carotid arteries It was totally absent in the other parts of the arterial system showing chan es

The media was very thin and the elastic tissue component was extensively destroyed The smooth muscle of the media had also to a great extent disappeared being replaced by collagenous connective tissue In the ascending aorta the aortic arch and at the origin of the left internal carotid artery the medial coat was replaced in places by a granulation tissue rich in lymphocytes plasma cells and capillaries (fig 3 B) The inflammatory cell reaction was strongest in the ascending aorta and the proximal parts of the aortic arch, there neutrophil granulocytes solitary eosinophil granulocytes and *multi nucleated* giant cells of foreign body type also occurred

The intima showed hyalin and fibrous thickening Typical atheromatous changes occurred mainly in the descending aorta. Only a weak, diffuse metachromasia was observed In the ascending aorta there occurred necrosis in atheromatous plaques with multi nucleated giant cells of foreign body type in connection with spaces left by dissolved cholesterol crystals

The obliterated portions of the brachial and axillary arteries showed a concentric narrowing of the lumen There was fibrous thickening at the intima and media, with destruction of the elastic tissue The adventitia showed fibrosis and an increase in the number of elastic fibrils There were no signs of inflammatory cell reaction

Other vessels With the exception of slight atheromatosis no pathological changes were observed

Other organs The histological examination verified the gross observations at the autopsy but yielded nothing further of interest

Diagnosis Takayasu s disease Hypertrophy of the left ventricle of the heart General congestion including acute and chronic congestion of the lungs Small old encephalomalacia Slight nephrosclerosis

Immediate cause of death Left sided heart failure

Discussion

The aetiology of Takayasu s syndrome is obscure Several authors (4, 7) have assumed that it is a kind of a hypersensitivity reaction in the vessel wall and have classified it as a collagen disease The presence of giant cells in the vascular wall is supposed to be due to a foreign body reaction to decomposed elastic tissue (7) But the giant cells may not be essentially connected with destruction of elastic tissue (8) The presence of giant cells has also been regarded as an indica-

tion of a primary affection of the connective tissue and a rheumatic aetiology (4) Previously a rheumatic aetiology has been discussed in our case (1, 9) At the post mortem examination we were unable to obtain any evidence of a rheumatic aetiology Nor was there any proof of a hyper sensitivity reaction, e g fibrinoid degeneration or fibrinoid necrosis The presence of giant cells and granulocytes in the ascending aorta and the aortic arch indicates however, an active process The marked fibrosis in the aorta, cervical and brachial arteries apparently represents the final stage of a previous pathological process From the clinical and histological picture it seems probable that the disease started in the large arteries in the neck and in the descending aorta and upper abdominal aorta The disease then progressed to involve the whole thoracic aorta The aetiology and the pathogenesis of the disease are not clear There is however nothing to contradict the hypothesis of a hypersensitivity reaction in the vessel wall to some agent of unknown nature (4 7)

Extensive calcification in cases of Takayasu s syndrome demonstrated at routine X ray examination during life has been reported (5 11) The latter authors report 3 cases in which calcification was observed in the thoracic aorta from its departure to the diaphragm At autopsy one of these cases showed calcification in the abdominal aorta also to the level of the origin of the renal arteries and in the proximal parts of the large arteries in the neck The vessels showed atheromatous intimal changes Yamada et al (11) do not give any more detailed information, however as to where in the vessel wall they found the calcifications Birke et al (5) found in one case, examined with ordinary routine X rays during life, very marked calcifications in the thoracic and abdominal aorta, and supposed that the calcifications were situated in the media When examined *postmortem* with microradiological equipment, our case showed extensive calcifications in the affected parts of the aorta, particularly in the descending aorta The X ray examination of cross sections through the aortic wall showed that the calcifications were localized to the intima, which was markedly atheromatous Calcification was therefore a part of the arteriosclerosis which was probably secondary to a preceding inflammatory process in the adventitia and the media Thus the calcification does not seem to be directly related to the primary affection of the vessel wall

The prognosis in Takayasu s *syndrome* is unfavourable in the long run and death is generally caused by cerebral ischaemia heart failure or impaired renal function (1) In this case the patient developed hypertrophy of the heart and symptoms of cardiac insufficiency the immediate cause of death was left heart failure The hypertrophy of the left ventricle was probably a sign of hypertension Reduced elasticity of the wall of the arterial wind kettle and interference with the renal blood supply are two possibilities to explain the hypertension encountered in Takayasu s syndrome (2) In our case the renal arteries showed only very slight arteriosclerotic intimal thickening at their origins without narrowing of the lumen The kidneys

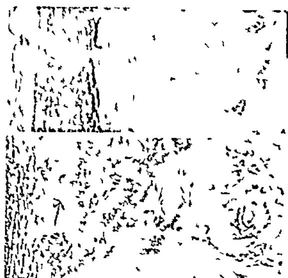


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The Effect of Varying the pH Level upon the Sensitivity of Urinary Bacteria to Antibiotics

By

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The results of current therapy in pyelonephritis are frequently disappointing (6 7, 12) Occasionally the failure reflects a poor correlation between the testing of bacteria *in vitro* and the effect of the antibacterial agent *in vivo* Astonishingly little attention has been paid in the clinical literature to the profound influence of urinary pH upon the effectiveness of some antibacterial agents

Normally the pH range of body fluids keeps within narrow limits Urine is an exception with a wide range from 4 8—8 0 rising after meals and falling to a minimum during the night The daily average is around pH 6 0 Under pathological conditions such as urinary infection caused by urea splitting *Proteus* species, we have observed a urinary pH of up to 9 0 Partial impairment of renal function frequently results in a corresponding loss of the ability to acidify urine (pH > 5 3) Thus under pathological as well as normal conditions the range of urinary pH is considerable Within this range of pH the activity of many drugs may vary markedly Routine testing of sensitivity to antibiotics by the disc method on solid media is generally performed either at constant pH or with complete disregard of the true pH of the medium This may account for the discrepancy between bacteriological tests and clinical experience

The purpose of the present investigation has been to develop an easy method for rapid determination of the optimal pH range for an antibacterial agent acting upon a particular bacterial strain isolated from the urine of patients with urinary infection Furthermore we have sought to investigate the triangular relation between antibacterial agent bacterial species and pH value

Alkalinization of the urine can usually be accomplished by oral administration of sodium bicarbonate Acetazolamide gives a more rapid effect (7, 22) Ammonium chloride and methionine give satisfactory acidification of urine (7 22 25 33) Pre existing renal in

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were of normal size and showed only slight nephrosclerosis Thus, the renal factor does not seem to be important in the pathogenesis of hypertension in our case It seems, however, likely that the hypertension is to some extent linked to an impaired "wind kettle" function of the aorta

Summary

The autopsy findings in a 47-year-old woman with Takayasu's syndrome are reported The clinical history of this case has been published previously

The changes occurred in the aorta from the aortic root down to the level of the departure of the renal arteries, in the proximal parts of the large vessels in the neck, and in the proximal parts of the brachial arteries The histological picture was characterized by marked adventitial and periadventitial fibrosis, destruction of the tunica media and atheromatous intimal changes Further, the ascending aorta and the aortic arch showed in the adventitia and media a strong inflammatory-cell reaction with the presence of giant cells of foreign-body type The arteries were examined for calcifications with microradiographical equipment Calcifications occurred as a part of the arteriosclerosis, which was probably secondary to the preceding inflammatory process in the adventitia and media and apparently represents the final stage of the previous disease The histological picture in the ascending aorta and the aortic arch, however, indicates an active inflammatory process The aetiology is obscure, but the histological picture does not contradict the possible occurrence of some hypersensitivity reaction of the vessel wall There was hypertrophy of the left ventricle, probably a sign of hypertension The immediate cause of death was left heart failure

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allowed to take place at room temperature (+ 20° C) for three hours before incubation at + 37 C for 15—18 hours The antibiotic discs employed are listed in table V

The assay of bacterial sensitivity to antibiotics was carried out in the manner described by H Ericsson et al (9 10) He also manufactured the discs which are supplied in Finland through A/B Distra Helsingfors Four degrees of sensitivity were recognized group I sensitive group II fairly sensitive group III slightly sensitive and group IV resistant

Control tests were in some instances carried out in buffered nutrient broth parallel to those on solid media The results obtained in liquid media confirmed those found by testing on agar plates This excludes the possibility of supposed pH effects really ascribable to altered diffusion properties in the agar due to different pH values

The reproducibility of the method The standard error of the method has been evaluated on the basis of duplicate determinations of the diameter of the inhibition zone of polymyxin The readings were made to one mm accuracy *E coli Klebsiella* and *Pseudomonas* were used in the test The range of the inhibition zone in this series was 12—20 mm the average 16 10 mm Variance analysis was applied The standard error of a single determination was found to be σ 0 82 *mm* (*with* 62 degrees of freedom)

The nine other antibacterial agents listed in table V were tested in complete duplicate determinations at the pH levels mentioned The parallel determinations were performed independently by two different technicians The distribution of the parallel observations is tabulated in table VI Technician A overestimates in 22 observations i e 4 79 per cent and technician B does the same in 21 observations i e 4 57 per cent. In a total number of 459 observations there consequently occurred only 9 36 per cent inconsistent estimates and they were equally distributed on both sides A and B agreed in 416 observations i e 90 63 per cent

Analyzing the causes of variations in his disc method Ericsson found a total standard

TABLE IV The composition of the liquid culture medium

Peptone (Evans)	10 g
Hepamino (Evans)	1 g
Beef extract	3 g
NaCl	3 g
Yeast autolysate	0 6 g
Water	ad 1 000 ml

TABLE V The antibiotic discs employed

Benzylpenicillin	20 I U
Ampicillin	13 4 g
Chloramphenicol	30 g
Chlortetracycline	50 g
Oxytetracycline	50 g
Streptomycin	50 g
Kanamycin	5 g
Polymyxin	50 g
Nitrofurantoin approx	30 g
Sulphathiazole	2 4 mg

TABLE VI The distribution of 459 parallel determinations of antibacterial sensitivity groups

TECHNICIAN B'S OBSERVATIONS \ TECHNICIAN A'S OBSERVATIONS SENSITIVITY GROUPS	IV	III	II	I
IV	156	5	0	1
III	10	78	1	4
II	1	1	19	10
I	5	0	5	163

TABLE I The distribution of the bacterial strains among the various species

Escherichia coli	131
Proteus subspecies	62
Pseudomonas aeruginosa	48
Klebsiella	29
Enterococcus	46
Staphylococcus aureus	22
Total	338

TABLE II The composition of the solid culture medium

Beef extract	3 g
Yeast autolysate	0 6 g
Agar agar (New Zealand)	13 g
Water	ad 1,000 ml

TABLE III The buffer salts employed at the desired pH levels

Obtained pH	$Na_2HPO_4 \cdot 2H_2O$	KH_2PO_4
5 5	0 475 g	8 72 g
7 0	7 24 g	3 54 g
8 0	11 22 g	0 50 g

sufficiency with hypernatremia or acidosis limits the use of these substances With intact renal function they may be regarded as relatively harmless

Methods and material

Bacteria Pathogenic bacteria from the urine of patients with persistent urinary tract infection were isolated and tested The patients were mostly urological cases Mixed infection occurred The distribution of the bacterial strains according to species is given in table I This distribution does not reflect the occurrence of various bacterial species in an unselected population with urinary tract infection Owing to the specialities of the hospital — urology and nephrology — patients with persistent and recurrent bacteriuria are referred to us from other centres

Preparation of buffered agar and broth The solid agar, upon which bacterial sensitivity was tested, had the composition given in table II After the preparation of the agar the buffer salts given in table III were added to the boiled agar mixture

The buffered broth was prepared with the same additions of buffer salts to a standard nutrient broth the composition of which is given in table IV

Fresh buffered agar and broth maintained its pH during autoclaving and incubation at + 37 ° C for 18 hours within 0 05 — 0 15 pH units Incidentally growth media were found to have a marked buffering action in counter acting pH changes

Determination of bacterial sensitivity Pathogens from mixed bacteriuria were first separated by surface streaking on standard agar plates (pH 7 2—7 4) Bacterial suspensions of sufficiently constant density were prepared as follows

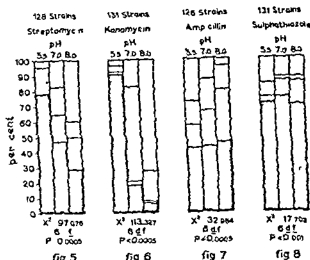
a) *Staphylococci* and *Enterococci*

A platinum loop was moistened in ten bacterial colonies The loopful was then transferred to a tube of 1 ml buffered broth and mixed thoroughly One drop of this mixture was then added to a second tube of 5 ml buffered broth and well mixed The agar plate was thereafter flooded with 5 ml of the prepared suspension

b) *E coli Pseudomonas aeruginosa Klebsiella* and *Proteus*

A 5 ml bacterial suspension was prepared as above (a) One drop of this suspension was further diluted in 5 ml of buffered broth after which the buffered agar plate was flooded with this diluted suspension

Excess of bacterial suspension was removed from the plate which was then left to dry for 30 minutes at + 37 C in a vertical position in a rack The antibiotic discs were then carefully set in position on the dried plates Pre-diffusion of the antibacterial agent was



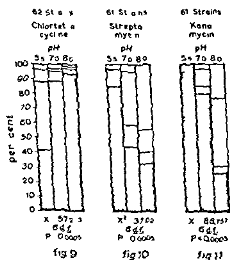
Figs 5—8 The antibacterial effect upon E. coli at three different pH levels.

deviation of $\sigma = 0.86$ mm for polymyxin with a mean diameter zone of 12.4 mm (9). On the basis of the two tests series presented above the method used can be regarded as sufficiently reliable for the purpose of the study.

These laboratory tests have mainly been performed at the hospital laboratory (Head R. Grasbeck M. D.

Results

Statistically significant effects of pH variations were found in the antibiotic-bacteria combinations listed in table VII. In each combination where a significant pH effect was found, there also occurred strains uninfluenced by



Figs 9—11 The antibacterial effect upon Proteus at three different pH levels.

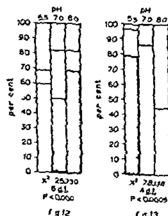


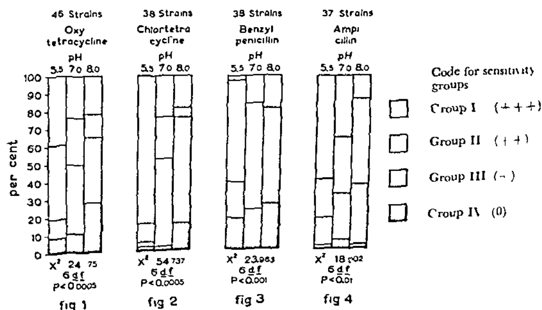
Fig 12 The effect of pH on the activity of chlortetracycline vs 22 strains of Staphylococcus aureus.

Fig 13 The effect of pH on the activity of kanamycin vs 29 strains of Klebsiella.

TABLE VII The influence of pH upon antibacterial action in combinations of drug vs bacterial species

Increased antibacterial effect at	Antibacterial agent						
	G-penicillin	Ampicillin	Oxytetracycline	Chlortetracycline	Streptomycin	Kanamycin	Sulphathiazole
(a) falling pH	Enterococcus [1](39%)	E coli (53%) Enterococcus (16%)	Enterococcus (41 3%)	Proteus (42%) Staph aureus (27%) Enterococcus (18 4%)			E coli (67%)
(b) rising pH					E coli (26%) Proteus (33%)	E coli (8%) Proteus (26%) Klebsiella (7%)	

[1] The percentage given in brackets refers to strains uninfluenced by pH variations



Figs 1—4 The antibacterial effect upon Enterococcus at three different pH levels

Increased antibacterial activity of benzylpenicillin with falling pH has been reported by numerous authors, although the results are somewhat conflicting (1 8 13) Robinson and Stevens found a tenfold greater activity of ampicillin at pH 5 5 than at 8 0 when testing strains of *E coli* and *Enterococcus* With *Staphylococcus* and *Proteus mirabilis* they found no significant effect (18)

Oxy and chlortetracycline Both antibiotics howed an increased effect upon *Enterococcus* with falling pH A similar but stronger effect of chlortetracycline was seen upon *Proteus* and *Staphylococcus aureus*

Eagle et al (8) found that the antibacterial activity of oxytetracycline at different pH levels varied markedly with the test bacteria The insolubility and instability of the tetracyclines in alkaline conditions the streptococcal preference for alkaline media and the production of alkalinity by the proteus group partly explain the favorable results in acid conditions in a large test series

Strepto and kanamycin The greatly increased effect of streptomycin in alkaline conditions has been confirmed by various authors (1 8 32) Our findings coincide with theirs but the effect occurred only with *E coli* and *Proteus*

In this study the results of strepto- and kanamycin are not wholly comparable because of the difference in amount of antibiotic in the discs A smaller amount of kanamycin is used on account of its greater toxicity The minimum inhibitory concentration of kanamycin is known to fall with rising pH although to a lesser degree than in the case of streptomycin (14) In this study the concentration threshold between groups IV and III is considerably greater for streptomycin than for kanamycin The thresholds between groups III and II and between II and I are, however, of the same magnitude In spite of the concentrations differences alkalinity has a strikingly higher effect upon kanamycin than upon streptomycin On the basis of this study we may expect a total resistance of proteus and almost total resistance of coli to kanamycin in the presence of acid urine Klebsiella is of interest because with streptomycin it shows a high resistance (50 %) and no significant pH effect whereas no kanamycin resistant strains are found at alkaline pH values

The risk involved in kanamycin and to a lesser degree in streptomycin therapy in impaired renal function is well known (3 4 5 11 15 24) Whether alkalization of urine prior to the institution of kanamycin as well as streptomycin therapy is imperative has to be further investigated in a clinical study This laboratory phenomenon however might explain the dramatic success of kanamycin therapy that is sometimes seen in urinary infections caused by urea splitting alkalizing proteus strains

Sulphathiazole A pH effect was seen with sulphathiazole acting upon *E coli* Measured with groups I and II the drug has a higher antibacterial activity in an acid medium The number of resistant strains was largest at neutrality

The pH effect within the bacterial species It is well known that bacteria can change the pH of their suspending medium The alkalinization of urine by

pH variations Their frequency is given in brackets The significance of the pH effect upon the behaviour of all the tested strains within each species was calculated

The significant combinations are presented as proportional bar diagrams in figures 1—13 Variance analysis was applied to the collected data and the significance examined by the chi square test Only the threshold between sensitivity group IV (i e resistant) and the sum of groups I—III was statistically analyzed The statistical data are found underneath each diagram

Discussion and comments

Many of the current theories concerning the effect of pH upon antibacterial agents complement each other The antibacterial action of basic substances (e g streptomycin) has been found to increase with rising pH, while that of acidic substances (e g penicillin) decreases This has been clearly established for streptomycin (1, 23) Conflicting results, however, have been obtained with penicillin (1, 13) The enhanced effect of streptomycin in alkaline conditions has been attributed to the greater permeability of the bacterial cell to undissociated molecules In some instances the effect of varying pH has been thought to be exerted mainly upon the receptor groups of the bacterial cell (2, 13, 16, 20, 21)

The present study does not disagree with these theories of a presumed "triangular" relationship between varying pH, bacterial cell and antibacterial agent, although the role of the culture medium should not be ignored in this connexion The most spectacular effect of pH upon a drug in our series can be seen with kanamycin and streptomycin The same effect does not, however, occur among staphylococci, enterococci and pseudomonas or, in the case of streptomycin, with Klebsiella Furthermore, in every combination where a significant pH effect was found there was for some strains no influence of pH despite a significant overall effect within the same species These uninfluenced strains occurred in the kanamycin and streptomycin combinations mentioned, either entirely in group IV (i e resistant) or, as in the case of kanamycin vs coli, only in groups IV and I In these cases the pH effect possibly occurred outside the range of the antibiotic concentrations represented by the sensitivity groups In all other combinations, however, except chlortetracycline vs enterococci, uninfluenced strains also occurred in groups II and III

Benzylpenicillin and ampicillin Both these antibiotics showed a significant pH effect with *Enterococcus* If the thresholds between groups II and III as well as between I and II were to be calculated statistically the significance of the effect would be stronger Furthermore, the antibacterial effect definitely increases with falling pH as measured with sensitivity groups I and II The effect can partly be explained by the optimal growth conditions of streptococci at pH 7 4—7 6 (19) The same holds for the pH effect upon the combination ampicillin vs coli

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Proteus has been mentioned We have not attempted to analyze this phenomenon separately, but rather have regarded it as a quality of the particular bacteria inseparably present *in vivo* as well as *in vitro* Attempts to acidify urine during an alkalizing proteus infection are, in our experience, usually unsuccessful Drugs that have their optimal effect in acid conditions are rendered ineffective

Enterococcus Only antibiotics with an optimal effect in acid media show a significant pH effect Alkalization does not lessen the high resistance (about 90 %) to kanamycin and streptomycin

E coli and Proteus E coli grows best at pH 7 4 and *Proteus* can stand high pH levels In both groups the pattern of the pH response of the antibiotic seems to dominate

Within the staphylococcus and klebsiella species there occurred only one antibiotic-bacteria combination with a significant pH effect This prevents conclusions about trends within these species

The antibacterial effect of benzyl penicillin, ampicillin, oxytetracycline and sulphathiazole seems to increase in acid conditions, while streptomycin and kanamycin are most active in alkaline media Kanamycin is almost without effect at acid pH values In each of these combinations, however, strains uninfluenced by the pH variations occur, which makes it difficult (except perhaps for kanamycin and streptomycin) to predict the effect of altered pH Rational antibacterial therapy of urinary infection presupposes that the optimal pH for antibacterial action upon the causative bacteria is determined and that attempts are made to adjust the urinary pH to this level The correlation between this phenomenon *in vitro* and *in vivo* has to be further clarified by clinical investigations Some of the laboratory results are, however, so striking that it seems justified to recommend determination of bacterial sensitivity to antibiotics at certain pH levels over a wide range as a routine laboratory procedure

Summary

A reproducible method for determination of the pH effect upon combinations of antibiotics vs bacteria is presented It is based upon Ericsson's disc method for determination of bacterial sensitivity to antibacterial agents Urinary bacteria were tested with benzylpenicillin, ampicillin, chloramphenicol, chlortetracycline, oxytetracycline, streptomycin, kanamycin, polymyxin, nitrofurantoin and sulphatiazole In certain combinations a statistically significant pH effect was found

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Studies on the Absorption Rate of Barbiturates in Man

By

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Only a few investigations on the absorption of barbiturates in man have been published (1 2, 3) Interest has been mainly focused on the elimination of barbiturate in plasma and to a lesser degree on the absorption pattern of barbiturates Little is known if any differences exist between the absorption rates of various barbiturates

In the present investigation the absorption of some intermediate and short acting barbiturates has been studied, as well as the influence of different dosage forms on the absorption

Methods

Healthy volunteers 20—25 years of age were chosen for the study The doses were administered in the morning on an empty stomach after fasting overnight No food or drink was allowed during the four hours of investigation Each time the substance was given with 100 ml of water The subject rested during the whole study Blood samples were withdrawn through an indwelling plastic needle in an antecubital vein immediately before and 15 30 60 90 120 150 180 210 and 240 minutes after the administration of the barbiturate Each time about 15 ml blood were drawn into heparinized test tubes and centrifuged The plasma was stored at —20 C until analyzed

Materials

The barbiturates investigated were amobarbital aprobarbital butabarbital cyclobarbital calcium cyclopentobarbital (= cyclopentenyl allyl barbituric acid) cyclopentobarbital sodium hexobarbital hexobarbital sodium pentobarbital sodium secobarbital and secobarbital sodium

The active substances were administered in hard gelatin capsules (Parke Davis and Co) in suspension in solutions or as tablets The capsules and suspensions of the sparingly soluble barbiturates were made with exceedingly fine powders prepared by micronization or precipitation with the exception of amobarbital and aprobarbital which were used in the ordinary commercial form The capsules and the suspensions contained 3 mg of sodium lauryl sulphate to improve wetting of the substances Capsules containing pentobarbital dissolved in polysorbate were prepared by gently warming 200 mg of the barbiturate in 800 mg of polysorbate 80 (Tween® 80)

The resultant solution was dispensed in hard gelatin capsules The capsules disintegrated

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TABLE II Plasma concentrations after administration of barbiturate salts in hard gelatin capsules

Substance	Weight of patient (kg)	Plasma levels after hours 1/4	1/2	1	1 5	2	2 5	3	3 5	4
Cyclobarbital	78	0 4	0 5	1 4	2 8	3 3	3 8	3 4	3 1	2 7
calcium 216 mg	60	0 4	1 9	3 0	4 0	4 2	4 2	3 7	3 1	2 7
Cyclopentobarbital	76	1 1	2 7	3 1	2 6	2 6	2 4	2 3	2 0	1 3
sodium 218 mg	71	2 8	4 1	4 3	3 6	3 4	2 8	2 2	2 2	1 7
Hexobarbital	52	1 4	7 0	5 8	6 3	5 7	5 5	4 2	3 5	2 3
sodium 546 mg	82	0 3	5 1	9 0	2 9	2 4	2 1	2.0	1 8	1 3
	70	5 9	5.9	5 8	4 9	3 5	4 0	2 8	3 0	2 4
Pentobarbital	67	1 2	3 2	3 5	3 8	3 3	3 1	2 6	2 1	1 4
sodium 220 mg	60	1 3	2 2	3 0	3 4	3 6	3 6	2 9	2 1	1 6
Secobarbital	71	2 4	3 8	2 6	1 7	1 8	1 5	1 6	1 8	1 2
sodium 220 mg	74	0 1	1 0	2 2	2 1	2 0	1 5	1 7	0 7	0 4

TABLE III Plasma concentrations after administration of 200 mg of barbiturate suspended in water or the corresponding amount of sodium salt in solution

Substance	Weight of patient (kg)	Plasma levels after hours 1/4	1/2	1	1 5	2	2 5	3	3 5	4
Pentobarbital	60	1 4	2 0	4 0	3 7	3 4	3 4	2 9	2 5	2 0
(suspension)	50	1 7	3 2	4 4	3 6	3 3	3 3	3 4	3 4	3 2
Secobarbital	75	0 5	0 5	1 1	2 8	3 3	2 8	2 6	2 8	2 1
(suspension)	64	2 3	4 4	4 1	3 6	3 6	3 2	2 8	2.7	2 6
Cyclopentobarbital	64	2 0	2 9	4 4	4 3	4 1	4 1	3 7	7 2	2 9
sodium (solution)	60	4 1	4 2	3 7	3 8	3 7	3 5	3 6	3 4	3 0
Pentobarbital	75	2 4	4 7	3 8	3 3	3 1	3 0	3 1	2.8	2 4
sodium (solution)	77	1 9	4 7	3 6	3 4	3 0	2 8	2 4	2 5	2.3
Secobarbital	61	1 6	4 9	4 4	3 4	3 2	2 6	2 7	2 4	1 9
sodium (solution)	56	5 8	4 8	4 0	3 3	2 9	3 0	2 8	2 5	2 4

increased absorption rate compared to capsules (table III) In particular the solutions gave a very rapid increase in the barbiturate concentration in plasma

Fig 1 summarizes the mean plasma levels of pentobarbital administered in various dosage forms The most rapid absorption was achieved by the solution of the sodium salt The acid form administered as capsules gave the slowest absorption The suspension of the acid, capsules and tablets of the salt formed an intermediate group The same tendency occurred in the trials with secobarbital and cyclopentobarbital and their salts

TABLE I Plasma concentrations after administration of barbiturates in hard gelatin capsules

Substance	Weight of patients (kg)	Plasma levels after hours ¼	½	1	1 5	2	2 5	3	3 5	4
Aprobarbital	62	1 3	1 7	2 0	2 9	2 6	2 6	2 0	2 0	2 0
200 mg	51	0 7	1 7	2 8	2 7		1 9			1 4
Butabarbital	77	0 2	1 8	2 9	3 5	3 6	2 9	2 6	1 8	1 7
200 mg	76	0 2	1 7	3 4	3 2	3 0	3 0	2 7	2 7	1 8
Cyclopentobarbital	62	0	0 2	0 6	0 7	1 6	3 1	4 1	3 2	2 9
200 mg	67	0 3	0 7	1 3	2 8	3 3	3 2	2 4	1 7	1 9
Hexobarbital	75	3 0	3 9	5 2	4 2	2 5	2 2	2 7	2 8	2 1
500 mg	82	0	1 1	1 8	2 9	3 6	4 2	5 2	4 8	3 9
	52	0 3	2 0	2 9	4 2	5 2	5 9	6 1	5 3	4 8
Amobarbital	70	0	0	1 4	1 8	2 4	2 0	1 9	1 9	1 4
200 mg	75	0	0 3	1 6	2 5	2 7	2 7	2 2	2 2	2 0
Pentobarbital	71	0 1	0 4	1 5	2 6	2 8	2 4	2 5	2 1	1 8
200 mg	79	0 4	0 8	1 6	2 4	2 8	2 7	2 8	2 9	2 4
Pentobarbital	58	0 2	1 1	2 9	3 0	2 9	2 5	2 0	1 9	2 0
200 mg dissolved in Tween®	53	0 2	1 5	2 6	3 1	3 2	3 0	2 5	2 7	2 5

in vitro within approximately two minutes and the tablets containing the sodium salts of secobarbital and pentobarbital within 30—60 seconds

Assay method

The determination of barbiturate in plasma was made according to the method of Zaar and Gronwall (4) with following slight modifications More plasma was used (2 00 ml) and absorbed on 6 filter paper strips The chloroform phase containing the mercuric barbiturate complex was separated by a capillary siphon eliminating the filtration step Corrections were made for the absorption obtained with the initial plasma sample

The accuracy of the individual determinations was approximately ± 35 per cent at concentrations less than 1 5 μg/ml ± 10 per cent in the range 1 5—3 0 μg/ml and ± 5 per cent relative standard deviation at higher concentrations Two determinations were made on each plasma sample and the mean values are given in the tables The method is probably not specific for unmetabolized barbiturates but may include metabolites with the barbituric acid ring intact

Results

When given as gelatin capsules in doses of 200 mg there was no apparent difference in the plasma concentrations of different barbiturates in acid form (table I) Hexobarbital was given in a higher dose (500 mg) because the analytical sensitivity of hexobarbital is half that of the other barbiturates

The corresponding doses of the sodium salts of barbiturates were absorbed more rapidly than the acid forms, but there was no apparent difference between the sodium salts regarding absorption rate (table II)

Distribution of the active substance in water before administration gave an

TABLE II Plasma concentrations after administration of barbiturate salts in hard gelatin capsules

Substance	Weight of patient (kg)	Plasma levels after hours 1/4	1/2	1	1.5	2	2.5	3	3.5	4
Cyclobarbital	78	0.4	0.5	1.4	2.8	3.3	3.8	3.4	3.1	2.7
calcium 216 mg	60	0.4	1.9	3.0	4.0	4.2	4.2	3.7	3.1	2.7
Cyclopentobarbital	76	1.1	2.7	3.1	2.6	2.6	2.4	2.3	2.0	1.3
sodium 218 mg	71	2.8	4.1	4.3	3.6	3.4	2.8	2.2	2.2	1.7
Hexobarbital	52	1.4	7.0	5.8	6.3	5.7	5.5	4.2	3.5	2.3
sodium 546 mg	82	0.3	5.1	9.0	2.9	2.4	2.1	2.0	1.8	1.3
	70	5.9	5.9	5.8	4.9	3.5	4.0	2.8	3.0	2.4
Pentobarbital	67	1.2	3.2	3.5	3.8	3.3	3.1	2.6	2.1	1.4
sodium 220 mg	60	1.3	2.2	3.0	3.4	3.6	3.6	2.9	2.1	1.6
Secobarbital	71	2.4	3.8	2.6	1.7	1.8	1.5	1.6	1.8	1.2
sodium 220 mg	74	0.1	1.0	2.2	2.1	2.0	[illegible]	1.7	0.7	0.4

TABLE III Plasma concentrations after administration of 200 mg of barbiturate suspended in water or the corresponding amount of sodium salt in solution

Substance	Weight of patient (kg)	Plasma levels after hours 1/4	1/2	1	1.5	2	2.5	3	3.5	4
Pentobarbital	60	1.4	2.0	4.0	3.7	3.4	3.4	2.9	2.5	2.0
(suspension)	60	1.7	3.2	4.4	3.6	3.3	3.3	3.4	3.4	3.2
Secobarbital	75	0.5	0.5	1.1	2.8	3.3	2.8	2.6	2.8	2.1
(suspension)	64	2.3	4.4	4.1	3.6	3.6	3.2	2.8	2.7	2.6
Cyclopentobarbital	64	2.0	2.9	4.4	4.3	4.1	4.1	3.7	7.2	2.9
sodium (solution)	60	4.1	4.2	3.7	3.8	3.7	3.5	3.6	3.4	3.0
Pentobarbital	75	2.4	4.7	3.8	3.3	3.1	3.0	3.1	2.8	2.4
sodium (solution)	77	1.9	4.7	3.6	3.4	3.0	2.8	2.4	2.5	2.3
Secobarbital	61	1.6	4.9	4.4	3.4	3.2	2.6	2.7	2.4	1.9
sodium (solution)	56	5.8	4.8	4.0	3.3	2.9	3.0	2.8	2.5	2.4

increased absorption rate compared to capsules (table III) In particular the solutions gave a very rapid increase in the barbiturate concentration in plasma

Fig 1 summarizes the mean plasma levels of pentobarbital administered in various dosage forms The most rapid absorption was achieved by the solution of the sodium salt The acid form administered as capsules gave the slowest absorption The suspension of the acid capsules and tablets of the salt formed an intermediate group The same tendency occurred in the trials with secobarbital and cyclopentobarbital and their salts

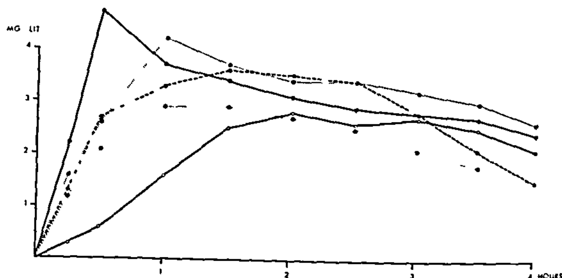


Fig 1 Plasma concentrations of pentobarbital after administrations in various dosage forms.
● ——— Pentobarbital sodium 220 mg solution
● ||||||||| Pentobarbital 200 mg suspension
● Pentobarbital sodium 220 mg capsules
● ///////// Pentobarbital sodium 200 mg tablets
○ ——— Pentobarbital 200 mg capsules

Some further trials were made to investigate the possibility of increasing the rate of absorption from solid dosage forms Capsules containing pentobarbital dissolved in Tween[R] 80, gave no definite improvement (table I) Neither did the addition of sodium bicarbonate to tablets containing the sodium salts of pentobarbital or secobarbital (table IV)

Rapidly disintegrating tablets of secobarbital sodium were administered to two groups of subjects One group (9 subjects) received the tablets in the ordinary way together with 100 ml of water and the other group (8 subjects) had them dissolved in 100 ml of water prior to ingestion Absorption was rapid for both methods of administration Significantly higher barbiturate concentration ($0.05 > p > 0.01$) was found 15 minutes after the administration of the dissolved tablets Thereafter no significant differences in the plasma concentrations of the groups could be found Four of the subjects receiving the solution showed maximum plasma concentration after 15 minutes The means and deviations of mean of the plasma concentrations are shown in figs 2 and 3

Discussion

For practical reasons it was not possible to use the same subject in a crossover design nor to use several subjects in every group However all studies were performed under strictly standardized conditions and apparently this technique allows detection of major differences in the absorption rate as can be seen from the size of the standard deviations in figs 2 and 3

TABLE IV Plasma concentrations after administration of pentobarbital sodium and secobarbital sodium in tablets

Preparation	Weight of patient (kg)	Plasma levels after hours								
		1/4	1/2	1	1 5	2	2 5	3	3 5	4
Pentobarbital	57	0 1	0 2	1 3	3 2	2 8	2 8	2 4	2 0	1 6
sodium 200 mg	62	1 1	2 1	3 5	2 9	3 2	2 4	2 1	1 7	1 6
tablets	70	2 5	3 9	3 8	2 7	2 0	2 2	1 8	1 6	1 3
Pentobarbital sodium	79	0 2	0 2	0 4	0 9	1 3	1 3	1 0	1 8	
200 mg + sodium bicarbonate 200 mg tablets	51	0 2	0 8	2 0	2 4	1 8	1 9	1 6	1 4	1 4
Secobarbital sodium	68	0 1	0 5	1 3	2 0	2 0	1 7	1 4	0 9	0 7
200 mg + sodium bicarbonate 200 mg tablets	60	0 9	2 7	3 1	2 2	1 5	1 3	1 0	0 9	0 6
Secorbarbital sodium	55	0 5	1 9	2 4	3 3	3 1	3 2	3 2	2 9	2 5
200 mg tablets	57		2 2	2 7	3 0	2 7	2 2	2 0	1 9	1 5
	58	0 3	1 2	2 0	2 0	2 2	2 2	1 8	1 4	1 3
	73	3 2	3 9	3 2	3 0	3 1	2 9	2 8	2 5	2 3
	71	1 8	3 1	3 2	3 0	3 2	2 9	2 5	2 6	2 0
	67	0 9	4 1	3 2	2 4	2 4	2 2	2 0	1 6	1 6
	73	0 7	2 2	2 8	3 1	3 0	2 9	2 5	2 3	2 0
	55	3 1	3 9	4 6	3 8	3 8	3 3		3 0	2 3
	70	3 0	4 7	3 4	3 2	2 7	2 3	2 4	2 5	2 2
Secobarbital sodium	80	2 4	4 0	3 4	3 2	2 9	2 8	2 6	2 5	2 2
200 mg tablets dissolved	50	1 9	4 9	3 7	3 4	2 9	2 5	2 3	2 0	1 5
in 100 ml of water before	71	4 7	3 7	3 2	2 7	2 8	2 3	2 3	2 2	2 2
administration	70	4 5	4 3	3 5	3 1	2 6	2 7	2 2	2 0	2 0
	72	3 5	3 9	3 1	3 2	3 0	3 0	3 0	2 5	2 2
	73	4 1	4 1	3 5	3 2	2 9	2 4	2 6	2 7	2 4
	75	4 5	3 5	2 3	1 8	1 8	1 9	1 8	1 7	1 4
	75	1 3	2 1	2 4	3 0	1 9	1 6	1 7	1 3	1 4

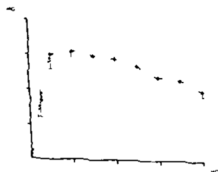


Fig 2 Plasma concentrations of barbiturate after administration of secobarbital sodium tablets (200 mg) Mean and deviation of mean from nine subjects

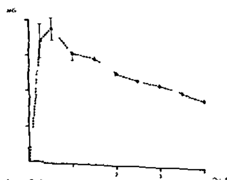


Fig 3 Plasma concentrations of barbiturate after administration of secobarbital sodium tablets (200 mg) dissolved in water Mean and deviation of mean from eight subjects

The concentrations of barbiturate in plasma were of the same magnitude as in previously reported studies using the same dose (1, 2, 3) However, the concentration maximum appeared earlier in our trials and this is likely to be due to different solution properties of the dosage forms used

It is not possible from this investigation to draw valid conclusions about the rate of elimination of the active barbiturate as the plasma concentrations were only followed for 4 hours and the analytical method is probably not specific for unmetabolized barbiturate All analytical methods hitherto used in barbiturate absorption studies in man appear to depend on an intact barbituric acid ring and are consequently non specific for unmetabolized barbiturate In absorption rate studies this source of error can be neglected, as the metabolites also must represent absorbed barbiturate

The acid forms of the barbiturates were absorbed more slowly than the sodium salts but no other absorption differences between different barbiturates could be demonstrated The very rapid absorption of solutions of sodium barbiturates indicates that the transport through the mucosa is not rate limiting for the absorption from the other dosage forms It appears that the rate limiting steps are the dissolution and the distribution of the substances in the gastrointestinal contents In spite of the rapid dissolution *in vitro* of the capsules containing barbiturate salts it is probable that the barbiturates are precipitated to a large extent by the gastric juice, and are then only slowly dissolved Apparently the same holds true for pentobarbital dissolved in Tween[8] and for tablets containing the sodium salts of pentobarbital and secobarbital When the tablets were allowed to disintegrate in water before ingestion the absorption was again very rapid and this indicates that the solution is spread in the gastrointestinal tract more efficiently and the risk of precipitation is less To obtain a rapid dispersion of the tablets in the gastric contents addition of sodium bicarbonate was tried but no effect could be demonstrated The generation of carbon dioxide from those tablets could not begin before the tablets were acidified and the barbiturate was precipitated The gas evolution was apparently insufficient to disperse the precipitate

A solution of a barbiturate salt thus seems to be the drug of choice in rapidly inducing sleep However, a solution has definite drawbacks such as limited stability and bad taste It is possible to circumvent the stability problems by using soluble tablets and dissolving them in water before use The disagreeable taste can make this mode of administration impractical in some patients but a fairly rapid absorption can still be obtained if tablets of the rapidly soluble type are swallowed with a glass of water

Summary

The absorption rate of some barbiturates after single oral doses have been studied in healthy volunteers The plasma concentrations were followed for 4 hours after administration No obvious differences were found in the absorption of the various barbiturates given in the same chemical form (salt or acid) in gelatin

capsules The salts regularly showed a more rapid absorption than the acids The most rapid absorption was obtained when the salts were given dissolved in water The dispersion and dissolution of the barbiturate in the gastro-intestinal tract seem to be the main factors determining the rate of absorption

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From the First and Third Departments of Medicine, University of Helsinki Helsinki Finland

Plasma Transport of Alimentary Triglycerides in Coronary Heart Disease

By

Risto Pelkonen and Esko A Nikkila

Post prandial hyperlipemia has been intensively studied in coronary heart disease since the original suggestion (28 29) that alimentary chylomicrons might be of importance in atherogenesis

The great majority of the studies in this field have revealed — regardless of the method used — the common occurrence of an abnormally high and prolonged post prandial hyperlipemia in patients suffering from coronary heart disease On the other hand parenterally administered artificial fat emulsions have been shown to disappear from circulation at similar rates in normal and coronary subjects (1 4 7 14 25 26 37) Nestel et al (31 32) have used human lymph chylomicrons and concluded that the rate of disappearance of these particles is chiefly dependent on the size of the endogenous triglyceride pool An increased endogenous plasma triglyceride pool i e an elevated basal triglyceride level in plasma is on the other hand a common finding in the coronary population as has been shown in a vast number of studies

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The mechanism underlying an abnormally high and prolonged post prandial hypertriglyceridemia may thus be centered on fat absorption, on the size of the liver plasma pool of endogenous triglyceride, or on the rate of removal of the lipid particles from circulation However the primary biochemical defects causing this abnormality have not yet been identified

The knowledge of the post prandial distribution of lipids in the different plasma lipoproteins in states of deranged lipid metabolism and in coronary heart disease is scanty (23) To fill this gap a study was made on the triglyceride and cholesterol content of ultracentrifugally separated plasma lipoproteins after a fat load in survivors of myocardial infarction and in healthy persons

Material

The material comprises 87 male and 8 female survivors of myocardial infarction 32 to 65 years of age and 57 healthy males and 21 healthy females between 19 and 65

TABLE I. Serum fasting triglyceride (mg/100 ml) and total cholesterol (mg/100 ml) levels in 9… survivors of myocardial infarction and 78 healthy persons

	Triglyceride			Cholesterol		
	N	Mean	SD	N	Mean	SD
Coronary	95	189.8	91.7	88	322.9	85.7
Healthy	78	98.1	34.5	72	237.3	37.2
Age below 35	43	89.1	33.8	39	235.7	36.0
Age over 35	35	108.8	29.0	33	239.2	36.7

years. In both groups the results of both sexes were pooled.

The group of survivors of myocardial infarction — in the text to follow briefly termed "coronary" — comprised patients all of whom had had a myocardial infarction 4 to 6 weeks before the study. The myocardial infarction was confirmed by electrocardiographic tracings. All patients with congestive heart failure, thyroidal disorders and manifest diabetes were excluded. At the time of the study the subjects were still hospitalized but already out of bed, and were receiving the ordinary hospital diet containing about 1,500 Cal and about 90 g fat per day. None were being treated by hypocholesterolemic agents or heparin, but most were on oral anticoagulants (phenindione).

The group of healthy subjects — in the following text briefly called "healthy" — included patients admitted to the hospital because of minor congenital heart disease without marked hemodynamic changes. In some subjects of this group the medical examination performed in the hospital did not reveal any organic disorder. In addition 6 healthy medical students were taken in the study. The medical students continued their dietary habit and the healthy patients received the usual hospital diet. Persons who showed serum cholesterol values over 350 mg/100 ml or serum triglyceride over 500 mg/100 ml, but who were otherwise healthy, were regarded as suffering from hypercholesterolemia or hyperlipemia and were not included.

Methods

The fat loading test was performed after an overnight fast of 12 hours' duration. In order to standardize the fasting period all subjects received a light meal exactly 12 hours before the fasting sample was taken.

A sample of venous blood was withdrawn in the morning and thereafter the subjects ingested 100 ml of cream containing 40 per cent of fat. Because vitamin A and E loading tests were done simultaneously, some of the subjects ingested in addition to the cream 1.5 million I.U. of vitamin A palmitate in 5 ml of soya bean oil equivalent to 5.9 mg of fat or 2 g of alpha tocopherol acetate in 10 ml of arachidis oil equivalent to 9.2 g of fat. The results of the vitamin loading studies have been published elsewhere (33, 35).

In the complete test the subjects were fasted for 6 hours after the fat load. Venous blood was drawn for serum triglyceride determinations 3, 4 and 6 hours after ingestion of fat. However, in 41 subjects only the fasting and the 4-hour samples were taken. Serum total cholesterol content was determined from the fasting samples only. In 25 coronary subjects and 41 healthy subjects plasma for lipoprotein lipid studies was taken in the fasting state and 4 and 6 hours after the fat load. Sodium EDTA was used as anticoagulant.

The lipoproteins were fractionated by flotation in a preparative ultracentrifuge (Spinco Model L) into D < 1.006 and D ˃ 1.006 sub fractions. The triglyceride

content was determined on all samples, but cholesterol content only in 9 coronary and 7 healthy subjects

The D > 1 006 fraction from 0- and 4-hour samples was fractionated further into D 1 006—1 019 D 1 019—1 063 and D > 1 063 sub-fractions in 12 coronary and 9 healthy subjects The triglyceride content was determined on all fractions in all subjects while the cholesterol content was determined in 9 coronary and 5 healthy subjects only

Triglyceride was determined by a combination of the procedures of Carlson and Wadstrom (10) and of van Handel and Zilversmit (19) and cholesterol by the method of Pearson et al (34) The isolation of plasma lipoproteins was performed essentially according to Havel et al (21) the details being as presented previously (35)

All analyses were performed in duplicate If the duplicates differed from each other by more than 10 per cent the samples were re analyzed

The recovery of triglyceride in the lipoprotein analyses showed a fairly great variability from 50 to 150 per cent However the samples in which the recovery was less than 60 per cent or exceeded 120 per cent were disregarded The recovery of cholesterol from the lipoprotein analyses varied considerably less and was on an average 85 per cent If the recovery was less than 75 per cent or more than 120 per cent the samples were disregarded

Results

Serum fasting triglyceride and cholesterol levels

As expected the coronary group had significantly higher mean basal triglyceride and cholesterol levels than the control group (table 1) The difference was also highly significant ($p < 0.001$) when the coronary group and the older healthy subjects (age over 35 years) were compared with each other Although this older healthy group had

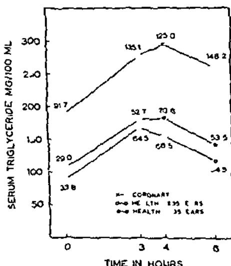


Fig 1 Serum triglyceride level in 95 survivors of myocardial infarction and 78 healthy subjects after ingestion of 100 ml of cream (40 per cent fat) Figures refer to standard deviations of the mean values

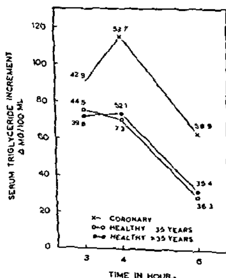


Fig 2 Serum triglyceride increment from the fasting level in 44 survivors of myocardial infarction and 28 young healthy subjects (age below 35 years) and in 26 old healthy subjects (age over 35) after ingestion of 100 ml of cream (40 per cent fat) Figures refer to standard deviations of the mean values

TABLE I Serum fasting triglyceride (mg/100 ml) and total cholesterol (mg/100 ml) levels in 95 survivors of myocardial infarction and 78 healthy persons

	Triglyceride			Cholesterol		
	N	Mean	SD	N	Mean	SD
Coronary	95	189 8	91 7	88	322 9	85 7
Healthy	78	98 1	34 5	72	237 3	37 2
Age below 35	43	81 1	33 8	39	235 7	36 0
Age over 35	35	108 8	29 0	33	239 2	36 7

years In both groups the results of both sexes were pooled

The group of survivors of myocardial infarction — in the text to follow briefly termed coronary" — comprised patients all of whom had had a myocardial infarction 4 to 6 weeks before the study The myocardial infarction was confirmed by electrocardio graphic tracings All patients with congestive heart failure, thyroidal disorders and manifest diabetes were excluded At the time of the study the subjects were still hospitalized but already out of bed, and were receiving the ordinary hospital diet containing about 1,500 Cal and about 90 g fat per day None were being treated by hypocholesterolemic agents or heparin, but most were on oral anticoagulants (phenindione)

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In the complete test the subjects were fasted for 6 hours after the fat load Venous blood was drawn for serum triglyceride determinations 3 4 and 6 hours after ingestion of fat However, in 41 subjects only the fasting and the 4-hour samples were taken Serum total cholesterol content was determined from the fasting samples only In 25 coronary subjects and 41 healthy subjects plasma for lipoprotein lipid studies was taken in the fasting state and 4 and 6 hours after the fat load Sodium EDTA was used as anticoagulant

The lipoproteins were fractionated by flotation in a preparative ultracentrifuge (Spinco Model L) into D $< 1\ 006$ and D $> 1\ 006$ sub-fractions The triglyceride

per cent level A correlation between the individual basal serum triglyceride values and the maximal post prandial triglyceride level was apparent in both groups (fig 3) The basal cholesterol level was not however correlated with the post prandial maximal increment of serum triglyceride

The ability of the fat loading tests to separate the coronary population from the healthy one was studied with as the dividing line the serum level which was not exceeded by 90 per cent of the young healthy subjects (from 19 to 35 years of age) In a previous study concerning a larger Finnish population these figures for fasting triglyceride and cholesterol were 125 mg/100 ml and 290 mg/100 ml respectively (35) The corresponding figure for 4 hour post prandial triglyceride — this point of time being chosen for practical reasons — calculated from the present data was 250 mg 100 ml With these concentrations as limits 40 per cent of the older healthy persons (age over 35 years) and 74 per cent of the survivors of myocardial infarction had an elevated serum fasting triglyceride level At 4 hours post prandially the upper normal limit of serum triglyceride level was exceeded by 17 per cent of the older healthy persons and by 62 per cent of the coronary group The respective percentages for cholesterol concentrations (in basal conditions) were 3 per cent for the older healthy persons and 63 per cent for the coronary group

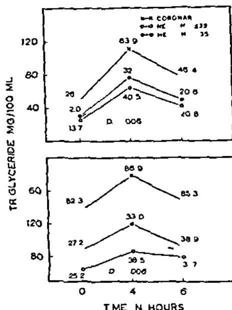


Fig 4 Triglyceride content of D < 1 006 and D 1 006 particles in 25 survivors of myocardial infarction and 23 young healthy subjects (age below 35 years and in 18 old healthy subjects (age over 35 years) after ingestion of 100 ml of cream (40 per cent fat) Figures indicate standard deviations of the mean values The dotted lines refer to the mean values for the whole healthy group Data corrected to 100 per cent recovery of the total serum content

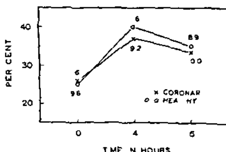


Fig 5 Triglyceride content of D < 1 006 particles as per cent of the total triglyceride content in 25 survivors of myocardial infarction and 41 healthy subjects after ingestion of 100 ml of cream (40 per cent fat) Figures indicate standard deviations of the mean values

Triglyceride distribution into lipoprotein particles

D 1 006 and D > 1 006 particles

In the primary separation (D <

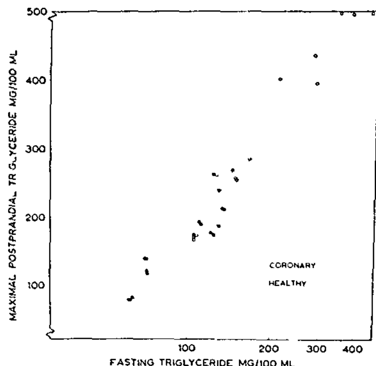


Fig 3 Relationship between serum fasting triglyceride and maximal post prandial triglyceride levels after ingestion of 100 ml of cream (40 per cent fat) in 47 survivors of myocardial infarction and 58 healthy subjects. The mean maximal post prandial triglyceride levels in the coronary group were 307 4 mg/100 ml and in the control group 189 4 mg/ml The lines show regression equations Coronary $x_0 = -847 \pm 522\ 9 \log x_1 + 52\ 2$ (p < 0 001) Healthy $x_0 = -604\ 4 \pm \log x_1 \pm 40\ 6$ (p < 0 001)

higher mean basal lipid levels than the younger one, the differences were not significant

Post-prandial serum triglyceride levels

The serum triglyceride concentration curves in figure 1 represent the mean values of all determinations Thus, the number of determinations varies at each point of time However, when these curves were compared with curves (not presented here) representing complete tests only, no real differences in the shape of the curves were found

The coronary group had the highest mean post-prandial triglyceride levels (fig 1) At each time the differences between the coronary group and the older healthy subjects were significant at the 0 1 per cent level The healthy subjects reached the peak level earlier than the coronary subjects Thus, the maximum level was reached at 3 hours by 46 per cent of the healthy persons but only by 19 per cent by the coronary group The respective percentages at 4 hours were 52 per cent for the healthy group and 67 per cent for the coronary group and at 6 hours 2 per cent for the healthy and 14 per cent for the coronaries In spite of the fairly parallel course of the serum post-prandial triglyceride concentration curves (fig 1), the absolute increment of serum triglyceride from the basal level was significantly higher at each point of time in the coronary group than in the healthy group (fig 2) The p values for inter group difference were 0 07 at 3 hours 0 001 at 4 hours and 0 02 at 6 hours

The maximal increment of serum triglyceride content was on an average higher in the coronary group (*130* ± 62 9 mg/100 ml) than in the healthy group (83 3 + 62 9 mg/*100* ml), the difference being significant at the 0 1

7), but the total serum triglyceride content did not correlate to the percentual distribution of triglyceride in these particles On the other hand an inverse correlation between the total cholesterol content and the percentual amount of triglyceride in the lighter particles was found to exist in the coronary group (fig 8) This was not the case in the healthy group Furthermore, the triglyceride content of both D < 1 006 and D > 1 006 particles in fasting sera seemed to correlate closely to the triglyceride level of unfractionated sera obtained 4 hours after fat ingestion The regressions were statistically significant in both groups (figs 9 and 10)

After fat ingestion the triglyceride content of both lipoprotein fractions increased in all subjects (figs 4 and 5) but relatively more in the light particles Essentially no difference between the coronary and healthy subjects was found in the relative distribution of triglyceride between these particles as judged from the very parallel course of the curves However, the post prandial triglyceride content of these particles (at 4 and 6 hours) was significantly higher (p < 0 01 — 0 002) in the coronary group than in the older healthy group The two healthy sub-groups did not differ significantly from each other

D 1 006 — 1 019 D 1 019 - 1 063 and D > 1 063

The further fractionation of D > 1 006 particles from the fasting samples and samples obtained 4 hours after fat ingestion was performed in 12 survivors of myocardial infarction and 9 healthy subjects

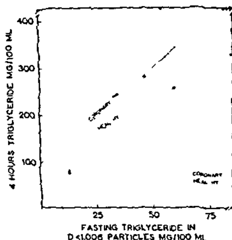


Fig 9 Relationship between fasting triglyceride content of D < 1 006 particles and the serum triglyceride level 4 hours after ingestion of 100 ml of cream (40 per cent fat) in 22 survivors of myocardial infarction and 34 healthy subjects The lines refer to regression equations Coronary r = 0 75 y = 4 21x + 65 9 (p < 0 001) Healthy r = 0 50 y = 2 75x + 92 2 (p < 0 01)

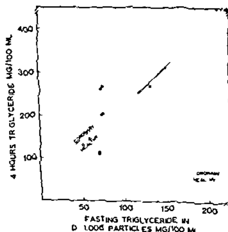


Fig 10 Relationship between fasting triglyceride content of D > 1 006 particles and the serum triglyceride level 4 hours after ingestion of 100 ml of cream (40 per cent fat) in 22 survivors of myocardial infarction and 34 healthy subjects The lines refer to regression equations Coronary r = 0 91 y = 1 62x + 65 9 (p < 0 001) Healthy r = 0 78 y = 1 85x + 33 4 (p < 0 001)

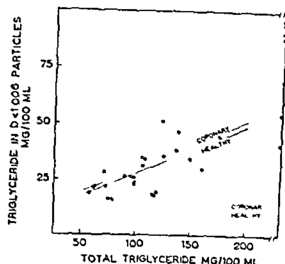


Fig 6 Relationship between the total triglyceride level and the triglyceride content of D < 1 006 particles in fasting sera from 24 survivors of myocardial infarction and 41 healthy subjects The lines refer to regression equations Coronary $r = 0\ 84$, $y = 0\ 21 + 8\ 9$ ($p < 0\ 001$) Healthy $r = 0\ 50$ $y = 0\ 20x + 8\ 6$ ($p < 0\ 001$)

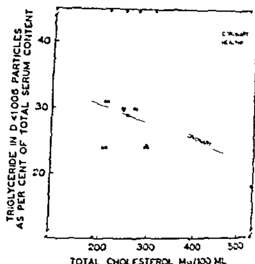


Fig 8 Relationship between serum total cholesterol level and the percentual distribution of triglyceride in D < 1 006 and D > 1 006 particles in fasting sera from 24 survivors of myocardial infarction and 39 healthy subjects. The line refers to the regression equation in the coronary group $r = -0\ 48$ $y = -0\ 02x + 35\ 8$ ($p < 0\ 02$)

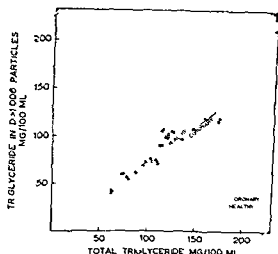


Fig 7 Relationship between the total triglyceride level and the triglyceride content of D > 1 006 particles in fasting sera from 24 survivors of myocardial infarction and 41 healthy subjects The lines refer to regression equations Coronary $r = 0\ 99$ $y = 0\ 79x - 8\ 9$ ($p < 0\ 001$) Healthy $r = 0\ 90$, $y = 0\ 76x - 2\ 5$ ($p < 0\ 001$)

1 006 and D > 1 006) a fairly fast speed of 21 000 r p m (28,360 × G) for 30 minutes was used Thus the D < 1 006 fraction contained the VLD particles in addition to chylomicrons

In fasting state on an average one-fourth of serum triglyceride was carried by the light particles in both the coronary and the healthy groups (figs 4 and 5) Thus, the amount of triglyceride contained in both particles was significantly higher in the coronary group than in the older healthy group, the differences being statistically significant ($p < 0\ 005$) Although the older healthy subjects showed a higher triglyceride content of both particles than the younger subjects, the differences did not have statistical significance

A close relationship existed ($p < 0\ 001$) in the fasting state between the triglyceride content of total serum and that of the D < 1 006 and D > 1 006 particles in both the groups (figs 6 and

TABLE II Total cholesterol content of D < 1 006 particles as per cent of total plasma content in 9 survivors of myocardial infarction and *7 healthy subjects after ingestion of 100 g of cream* (40 per cent fat)

Subject	Fasting		4 hours		6 hours		10 hours	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Coronary	13 7	10 – 19	15 1	12 – 22	15 5	10 – 20	16 6	9 – 23
Healthy	15 0	7 – 20	12 1	8 – 16	13 4	10 – 19	14 8	11 – 19

TABLE III Total cholesterol content of D 1 006—1 019 D 1 019—1 063 and D > 1 063 particles as per cent of D > 1 006 fraction in sera of 9 survivors of myocardial infarction and in 5 healthy persons after ingestion of 100 ml cream (40 per cent fat)

	Fasting		4 hours		6 hours		10 hours	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
D 1 006—1 019								
Coronary	15 5	9 – 27	17 6	4 – 32	23 7	10 – 49	22 3	12 – 32
Healthy	7 4	3 – 16	13 2	7 – 22	13 5	9 – 21	14 0	7 – 20
D 1 019—1 063								
Coronary	69 3	62 – 72	64 4	54 – 85	57 5	40 – 70	59 7	50 – 71
Healthy	73 5	67 – 80	65 8	65 – 71	65 6	62 – 72	61 2	59 – 64
D 1 063								
Coronary	15 2	11 – 21	18 0	11 – 28	18 8	11 – 31	18 0	11 – 27
Healthy	19 1	13 – 26	21 0	18 – 27	20 9	17 – 25	24 8	18 – 34

total serum was determined only from the fasting samples The 10 hour samples *were obtained after an ordinary hospital* dinner

About 15 per cent of serum total cholesterol was carried by D < 1 006 particles in the fasting state in both groups under study Fat ingestion did not influence substantially the distribution pattern (table II)

Almost all the D > 1 006-cholesterol was bound to the D 1 019 — 1 063 fraction in fasting serum (table III) The coronary group had relatively more cholesterol in the D 1 006 — 1 019 particles than the healthy group, whereas the opposite was found in the other particle classes of density more than 1 019 Only small changes were observed after fat ingestion The most marked change was the relative increase of cholesterol content in D 1 006 — 1 019 particles with a concomitant decrease in the class just above (D 1 019—1 063) The HD-cholesterol did not change post prandially

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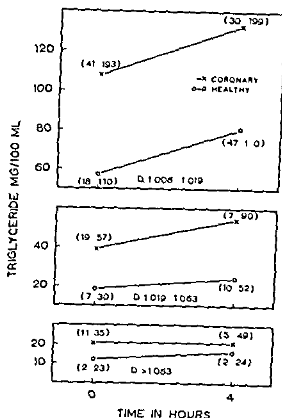


Fig 11 Triglyceride content of D 1 006—1 019, D 1 019—1 063 and D > 1 063 particles in 12 survivors of myocardial infarction and 9 healthy subjects after ingestion of 100 ml of cream (40 per cent fat) Figures refer to the range of the triglyceride amounts Data corrected to 100 per cent recovery of triglyceride content of D > 1 006 fraction

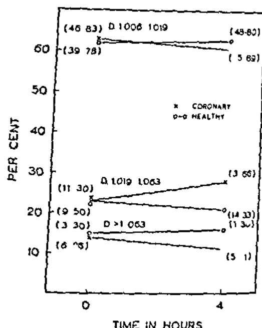


Fig 12 Triglyceride content of D 1 006—1 019, D 1 019—1 063 and D > 1 063 particles as per cent of total D > 1 006 fraction in 12 survivors of myocardial infarction and 9 healthy subjects after ingestion of 100 ml of cream (40 per cent fat) Figures show the range of the values

In the fasting state the greater part, equal to about 60 per cent of > 1 006 bound triglyceride, was carried by D 1 006 — 1 019 particles in healthy and coronary subjects (figs 11 and 12) Of the remaining part slightly more was bound to the D 1 019 — 1 063 fraction than to the HD particles (D > 1 063) The coronary group had in each lipoprotein class more (about 2 fold) triglyceride than the healthy group, but there was no difference in relative distribution pattern

After fat ingestion the triglyceride in D 1 006 — 1 019 particles rose in both groups, but that in the D 1 019—1 063 particles only in the coronary group No changes occurred in the triglyceride of HD particles (fig 11) The fat ingestion did not affect the distribution pattern substantially (fig 12)

Percentual distribution of total cholesterol in lipoprotein particles

The percentual distribution of total cholesterol in D < 1 006 and D > 1 006 particles was followed after fat ingestion in 9 survivors of myocardial infarction and 7 healthy subjects (table II), and that in D 1 006 — 1 019, D 1 019 — 1 063 and D > 1 063 particles in all but two healthy subjects (table III) The results are expressed as per cents only, since the total cholesterol content of

reported for the initial exponential removal in rats

In man the fractional clearance of labeled fat from blood seems to diminish with increase of total circulating triglyceride mass Thus, Berkowitz et al (5) showed that the disappearance rate of I^{131} tagged triolein was slower during alimentary lipemia than in the fasting state Likewise Bierman and Hamlin (6) reported that the removal rate of a C^{14} triglyceride preparation decreased with increasing fat load Nestel (31) has found with human lymph chylomicrons that the fractional removal rate of fatty acid label is inversely related to the fasting triglyceride level and to the intensity of alimentary lipemia produced by a constant fat load However the total amount of fat used in his tracer study was minimal (15 mg/kg) and, therefore provided that the chylomicrons completely share the fate of endogenous plasma triglycerides the results approximately reflect the turnover rate of plasma triglyceride fatty acids at a steady state condition Calculated from the data of Nestel (31) the actual turnover rates are revealed to increase from about 10 to 12 mg per minute per 100 ml plasma at a triglyceride concentration of 100 mg/100 ml to about 17 to 19 mg/min at a basal triglyceride concentration of 300 mg/100 ml Thus persons with hyperglyceridemia *can* extract considerably more triglycerides from blood than is needed for a normal turnover Of course the hyperglyceridemia may develop also due *to an actual removal* defect as exemplified by the rare lipoprotein lipase deficiency disease (22) In fact Gries et al (18) have divided human hyperlipemias into groups with normal and retarded elimination rates of labeled fat

In accordance with recent results by others (12, 20, 31, 32) *the present* study has revealed a close relationship between the basal triglyceride level and the maximal post prandial triglyceride increment This finding does not imply either a deficient or a fully saturated removal mechanism, but is best explained by the fact derived from the animal and human studies cited above that the elimination rate of triglycerides from plasma does not increase in pace with their concentration This indicates that the removal system has a relative limit of capacity at subsaturation levels The possibility that an elimination maximum is exceeded during alimentary lipemia in man cannot be ruled out with the data at present available

A defect in the conversion of Sf 10—100 to Sf 3—9 lipoproteins has been suggested by Gitlin et al (17) in nephrotic hyperlipidemia A specific fault in the metabolism of Sf 20—400 lipoproteins has been also suggested to underlie the pathologic post prandial lipemia in coronary heart disease (16) Studies on plasma transport of vitamin A showed also that the abnormal vitamin A tolerance commonly occurring in coronary patients was characterized by a post prandial retention of vitamin A bound to D < 1.006 and D 1 006—1 019 particles (35) Therefore the possibility of a specific error in the lipoprotein lipid transport system cannot readily be excluded when the abnormal post prandial lipemia is considered

The mere determination of concen

Discussion

The abnormally high and prolonged post-prandial lipemia commonly occurring in patients with coronary heart disease, particularly in cases with hyperglyceridemia, has been attributed to a faulty intestinal handling of neutral fat (24, 36), to an intensified absorption of fat (2), to a deficient removal of exogenous fat from circulation (8, 27) and to a mixing of the absorbed fat in a large endogenous triglyceride pool (12, 20, 32)

The plasma triglyceride level at any time point after fat ingestion is determined by the ratio between influx rate of fat from the gut and its removal rate from the blood If the sites and mechanisms for removal of exogenous and endogenous plasma triglycerides are identical, as seems to be the case, then alimentary fat imposes an extra load on the system already extracting the endogenous glycerides An unusually high post prandial increase of plasma triglyceride concentration must be attributed to either an increased influx rate or a decreased efflux rate or both An accelerated inflow is expected to cause a high peak level of plasma triglyceride but a fairly rapid return to pre-prandial value, a situation never demonstrated in cases with disordered lipid metabolism Thus, there could be an actually deficient removal mechanism of exogenous triglycerides from the circulation, or else an attainment of an absolute or relative maximum capacity of a *per se* normal extraction system of all plasma triglycerides The first alternative includes all true removal defects, i e, a decreased blood flow through the active sites and a deficient uptake or metabolization of triglyceride molecules (or particles carrying them) Ultimately the view may prevail that the primary clearing is normal but that recirculation occurs to an abnormally high degree.

The true elimination rate of exogenous triglycerides from the blood stream can be adequately measured only with intravenously administered native chylomicrons, although artificial fat emulsions are probably handled in a similar way (9, 13) In most studies the initial removal of chylomicrons has been of single exponential type both in animals (for review see Carlson and Hallberg) and in man (31, 32) The half life times have in general varied between 3 and 13 minutes French and Morris (15) and Edgren and Meng (13) have found the fractional disappearance rate of chylomicrons to be inversely proportional to the total amount of fat injected within the dose range 50 to 1,000 mg/kg However, the absolute quantity of fat removed per unit time in these experiments increased with increasing load On the contrary, Belfrage et al (3) could not demonstrate any change in the fractional disappearance rate of fatty acid labeled chylomicrons when the fat dose varied from 20 to 170 mg/kg Thus, the absolute efflux rate of fat from circulation was directly proportional to the lipid dose In the dog, Carlson and Hallberg (9) have demonstrated the presence of an elimination maximum for both chylomicron and artificial emulsion triglycerides, i e a plasma triglyceride concentration above which the removal proceeds at a constant (linear) rate This maximal rate was much lower than the highest figures

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D < 1 006 and D > 1 006 sub fractions The D > 1 006 fraction from fasting and 4 hour samples was fractionated further into D 1 006—1 019, D 1 019—1 063 and D > 1 063 sub fractions in 12 coronary and 9 *healthy subjects* The triglyceride content was determined from each subfraction

The coronary group had significantly higher cholesterol and basal triglyceride values and a higher post prandial tri glyceride level than the healthy group At each time point the significance of the difference was at the 0 1 per cent level Furthermore the coronary group showed a higher absolute increase and *maximal increment* of the plasma tri glyceride from the basal level than did the healthy group On an average the healthy subjects reached the maximal post prandial triglyceride level earlier *than the coronary group* There was a close interrelationship between the in dividual basal triglyceride values and the maximal post prandial triglyceride level in both groups

The *fat tolerance test* did not separate the coronary subjects from the healthy subjects better than did the basal lipid values

About one fourth of the plasma total triglyceride was carried by the lightest particles (D < 1 006) and about 60 per cent of the remainder was bound to the D 1 006—1 019 particles The percentual distributions of triglyceride in all these particles were equal in the two groups studied The triglyceride content of each fraction was highest in the coronary group

After the fat load the triglyceride content increased in particles of D < 1 006, D 1 006—1 019 and also, in the coronary group, in the D 1 019—1 063 particles The coronary group showed a higher increase than the control group in *all triglyceride fractions* In both groups the fasting triglyceride contents of D < 1 006 and D > 1 006 particles were closely related to the 4 hour total triglyceride content of plasma

About 15 per cent of the total plasma cholesterol content was carried by D < 1 006 particles Almost all the D > 1 006 cholesterol was bound to the D 1 019—1 063 particles The coronary group had relatively more cholesterol in the D 1 006—1 019 particles than the healthy group

Fat loading influenced only slightly the cholesterol distribution in the lipo-proteins

Acknowledgement

This work has been aided by grants from Sigrid Juselius Foundation Emil Aaltonen Foundation and Yrjo Jahnsson Foundation Finland.

Addendum

During the preparation of the manu script an extensive study on fat tolerance tests in hyperlipidemic subjects with and without atherosclerosis was published by G Angervall (Acta med scand Suppl 424, 1964) His results were in many respects in accordance with those of the present study A good correlation between the triglyceride con-tent of fasting serum and that of serum obtained 4 hours after a fat meal was found Interestingly, subjects without clinical atherosclerosis differed from

trations of unlabeled compounds — as was done in the present study — does naturally not permit any calculation of the flux rates However, the decline (from 4 to 6 hours) of the concentrations of total triglyceride and of the D < 1 006 and D > 1 006 particles showed a significant parallelism in the coronary and healthy groups Unfortunately the concentrations of the sub-fractions of D > 1 006 were followed for only 4 hours post-prandially, but the post-prandial lipoprotein determinations recently made by Harlan and Beischer (20) revealed that a similar parallelism occurs also in these subfractions

Thus, the present curves as well as the results obtained by Harlan and Beischer (20) suggest that the difference in the post-prandial hypertriglyceridemia found between the coronary and control groups was not due to any specific particle, i e incorporation of triglyceride into the particle or disposal of the particle from circulation

From the considerations presented above it follows that the same defect that causes an elevated fasting triglyceride level may also be responsible for the abnormal post-prandial lipemia Increased *de novo* synthesis of fatty acids has been suggested to underlie the carbohydrate-induced hyperlipemia, but has no experimental evidence In fat-induced hyperlipemia an increased influx of free fatty acids from adipose tissue has been thought to play an important role in the genesis of hypertriglyceridemia It has been postulated that the plasma FFA response to norepinephrine is accentuated in hyperglyceridemia (30), but on the other hand no difference has been found in this respect between normal and coronary subjects (11)

The correlation between fasting and maximal post prandial serum triglyceride levels was quite similar in healthy and coronary subjects Thus, in agreement with the conclusions made by Denborough and Nestel (12, 31), it may be stated that coronary heart disease is not accompanied by any abnormality in handling exogenous fat provided that the endogenous triglyceride metabolism is normal Accordingly, oral fat loading tests as performed in the usual way apparently are not superior to basal triglyceride determinations as a predictor of coronary heart disease Further more, unlike the situation in diabetes, very few "latent" cases of disordered triglyceride metabolism are expected to be detected by an oral fat tolerance test On suspicion of a real triglyceride removal defect, this procedure might be used as a simple screening test before performance of the more laborious turnover studies with intravenous labeled fat

Summary

A fat loading test with 40 g of cream fat was performed in 95 survivors of myocardial infarction and in 78 healthy persons The plasma triglyceride content was determined before and 3 4 and 6 hours after the fat load Plasma cholesterol was determined from fasting samples

In 25 survivors of myocardial infarction and 41 healthy subjects, the fasting and the 4 and 6 hour samples were fractionated by ultracentrifuge into

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those with atherosclerosis in respect of the turbidity in relation to triglyceride in serum before and 4 hours after the fat meal, patients with clinical atherosclerosis having lower turbidity than those without Since no difference was demonstrated between triglyceride in the chylomicron phase of these groups, it was concluded that there must be a difference in chylomicron size

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Renal Arteriography in Arterial Hypertension

By

ERIK ASK UPMARK and STIG FAGERBERG

In previous papers the presence of renal arterial obstruction as the cause of an arterial hypertension has been demonstrated by one of us (2 4, 5 7) Whereas it is tempting, in such instances, to ascribe the hypertension to the arterial obstruction along the lines indicated by Goldblatt (10 11) it has also been emphasized that arterial obstructions of the renal arteries may be present without any hypertension (7 12 18) Obviously the contrary is true as well since most instances of arterial hypertension are probably not due to any interference with the renal blood supply

The present paper will attempt an analysis in this regard of 62 instances of arterial hypertension observed in the Department of Medicine during the last few years (1963 1964 end of 1962 and first 3 months of 1965) and subjected to arteriography of the kidneys in the Department of Diagnostic Roentgenology Renal arteriography was as a rule carried out by means of a catheter passed up into the abdominal aorta from the femoral artery according to Seldinger s method

The indications for renal arteriography in arterial hypertension have mainly remained the more or less time honoured landmarks

1 Young age
2 Sudden onset of hypertension
3 Rapid malignant course of hypertension
4 Absence of hypertension in the pedigree
5 Attacks of hematuria
7 Different size of the two kidneys
8 Arterial bruit over renal hilus
9 Genital hypoplasia
10 Naevus flammaeus in the renal region
11 Polycythemia (14)
12 Presence of arterial stenosis elsewhere in the body
13 In females, cardiac enlargement not otherwise accounted for

However it may be said that examination by means of renal arteriography may sometimes also have to be under

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The *case d*) (fig 1) will be briefly described

Man aged 26 (Med Rec 37 04 28—841) Blood pressure 220/105 on occasional exam nations May 1963 In July 1963 BP 230/140—130 eye grounds KW 3 NPN 39 mg% concentration test 1016 heart size 390 ml/m² Split renal function test Sept. 1963 (PAH and inulin clearance catheters in renal veins) suggested reduced blood supply of right kidney Urography left kidney normal right not to be identified

Abdominal aortography left kidney two normal arteries Right kidney one artery which had its lumen reduced to a diameter of 1 mm for a distance of 0 5 cm Proximal to this stenosis an arterial branch takes off from the renal artery bound for the upper part of the kidney

The right kidney is smaller than the left one Nephrography delayed excretion lower pole right kidney

Aldosterone excretion registered Aug 1963 at the upper limit of the normal variation (the analyses were kindly carried out by Dr Holsfeldt Stockholm)

Serum potassium Sept 1963 3 1 ME/l

Surgery was performed twice by our colleague Professor Thorén

1) Sept 23 1963 *resection* of lower pole of right kidney no pulsations were felt in the arteries to this pole) with only moderate if any effect on the hypertension
2) Oct 31 1963 removal of the remaining kidney which was enclosed in cicatricial tissue necessitating the removal of the right suprarenal gland as well

Histological examination of the lower pole of the right hypoplastic kidney revealed tubular changes compatible with reduced blood supply

Histological examination of the rest of this kidney revealed an infarction like area in front of this upper pole of the kidney with very wide serpiginated tubuli obviously of malformational character (the so-called Ask Upmark kidney)

Subsequent course the patient was admitted for re-examination Dec 1963 Jan 1964 *and Sept 1964 Blood* pressure came down to 130 90 at the last examination not exceeding 150/95 General condition fine Eye grounds improved being classified as K W I Heart size remained normal Potassium level normalized (4 ME l)



Fig 2 Renal angiography in a man aged 42 who had *multiple arterial lesions* one of which was a stenosis and a post stenotic dilatation of the left renal artery This man had no increased blood pressure and is accordingly not included in the material here to be considered



Fig 3 Renal arteriography in a man aged 53 with a most severe arterial hypertension (eye grounds grade 4) The renal arteries in this case obviously normal radiologically

2 In 28 females (aged 19—69 average age 48 a positive *observation* was registered in 10 instances (aged 35—66 average age 44) whereas normal conditions were recorded in 18 instances (aged 19—68 average age 48)

The positive observations were

a In 5 instances one sided hypoplasia (aged 40 42 50 and 69) In one of these cases (the woman aged 69) there was an arterial stenosis as well a complete obliteration of



Fig 1 Renal angiography in a baker aged 26, who had arterial hypertension The stenosis of the right renal artery and the post stenotic dilatation are readily evident The case is described in the text as d) among the male cases Nephrectomy brought recovery Lesion Malformation of the so-called Ask Upmark type

taken in instances devoid of such features In a previous paper attention was thus called to a man, aged 67, in whom an arterial hypertension was precipitated by an embolus in the main renal artery on the left side, this kidney obtaining some degree of arterial supply from an accessory renal artery The removal of this kidney was followed by recovery with regard to the hypertension for the following 18 months (5) A mature age should accordingly not be considered a contraindication if there are other grounds for suspecting a reduced arterial renal supply as the responsible factor Generally, it may be said that with increasing experience our indications for this examination have been widened

Material

The material was represented by 62 instances of arterial hypertension, observed in our clinic In all of them were registered the family history, the level and if possible also the duration of the arterial hypertension, the appearance of the eye grounds (Keith Wagener), the renal functions, the size of the heart and its electrocardiography and the radiological anatomy of the abdominal aorta and its renal branches In several but not all instances such features as the catecholamine excretion, the serotonin, the 17 ketosteroids and occasionally the aldosterone production were recorded as well The various observations may be briefly summarized as follows

I In 34 males, aged 19—63, a positive observation was made by means of the abdominal aortography in 9 instances (aged 26—63, average age 52), whereas normal renal conditions were registered in 25 instances (aged 19—63, average age 45)

The positive observations were

a) In one case polycystic kidneys (aged 59)
b) In one case a renal tumor (aged 59)
c) In two instances one-sided renal hypoplasia
d) In one case a one sided renal hypoplasia in connection with reduced arterial supply to this kidney
e) In four instances arterial stenosis on one or both sides without any interference with the size of the kidney Only one of these cases was subjected to surgery (patch graft of the left renal artery) The operation was carried out on Feb 18th and has so far (one month later) not brought about any amelioration

It should be added that in one of the two cases (c) with one-sided hypoplasia of the kidney a certain narrowing of the artery was also observable

In the case with a renal tumor (b) the blood pressure before the operation was 270/150 whereas after the operation it was 160/100 the blood supply of this tumor was so abundant as to suggest almost the presence of some kind of shunt mechanism as if the otherwise normal renal artery really may have been too narrow for the demands of the normal renal tissue Hence a Goldblatt mechanism was presumably induced in much the same way as when an arteriovenous aneurysm of the brain occurs and is apt to reduce the arterial supply of the brain tissue resulting in a symmetric hydrocephalus internus

underline the need for caution in judging positive observation along these lines and the necessity for completing the evidence by means of other methods of examination for instance the split renal function test Donald Smith has devised a simplification of this test through confining the registrations to the specific gravity of the urine from each of the kidneys (19)

Notwithstanding the reserved judgment already mentioned we have to admit that there are instances where an arterial hypertension in human beings may be caused by interference with the renal blood supply As mentioned in an earlier paper by one of us, it seems reasonable to assume that the arterial hypertension so frequently encountered in the Takayasu syndrome may be caused by reduced blood supply to one or both of the kidneys (2) That also in other instances of arterial hypertension the renal blood supply may represent the responsible factor has been amply substantiated in numerous publications for instance in a recent paper from this clinic 5) as well as in the distinguished observations by Rosenheim and his collaborators at University College Hospital in London (16)

It is suggested in the present paper that an arteriovenous shunt of the kidney may be responsible for a relative ischemia of the renal cortex hence inducing a Goldblatt mechanism also in such instances

In the entangled problem of arterial hypertension the outstanding factors established include the following

1 Heredity
2 Suprarenal tumor (phaeochromocytoma Cushing Conn)
3 Reduced arterial volume (coarctation)
4 Malnutrition (constantly low blood pressure in instances of sprue, according to Salvesen of Oslo)
5 Acute increase in intracranial pressure, such as in subarachnoid hemorrhage
6 Renal disorders, anticipated perhaps already by the old Chinese medicine, and substantiated by Bright and especially, during the last decade, by Goldblatt and his distinguished work on the arterial blood supply

Summary and conclusions

1 Reduced arterial blood supply to the kidney may result in arterial hypertension along the lines indicated by Goldblatt as substantiated by numerous observations
2 There may be stenosis of one or more of the renal arteries that is not responsible for the encountered hypertension and there may exist stenosis of a renal artery without any hypertension
3 It is suggested that arteriovenous shunts in the kidney arising through pre formed malformations or by renal tumors may be responsible for the induction of a Goldblatt factor

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the lumen about 1 cm from the origin of the left renal artery from the aorta, whereas there was a strangulation of the right renal artery about 0.5 cm from its aortic origin.

b) In 4 instances (aged 35, 13, 44, and 59) arterial stenosis was observed in one or both of the renal arteries without any hypoplasia of the kidney.

In one case (the woman, aged 59, with an initial blood pressure of 250/135) both renal arteries were involved. In this case, however, there was also a suprarenal cortical hyperplasia which was operated upon, whereupon the blood pressure was normalized. In this case the clinical diagnosis was Cushing's syndrome. The surgical specimen disclosed a diffuse hyperplasia of the suprarenal cortex, in part nodular. In another case (woman, aged 35) both renal arteries were afflicted by a stenosis as well and so was the art. mesenterica superior. In this case the hypertension had been present since her first delivery at the age of 18. There was also a severe hypertension in the family, her mother and her maternal grand-parents having had a severe hypertension and one younger sister had died from arterial hypertension.

In one case a considerable narrowing of all arterial branches to one of the kidneys was observed. In this case however there was hardly any hypertension (180/110 reduced to 160/90 whilst in the hospital) and the cause of the reduced circulation of this kidney was an inveterated renal tuberculosis (the so called cement kidney, in German "Kittniere").

In one case, a female aged 40, an arterio-venous fistula was disclosed by the renal angiography and eventually confirmed by the bruit discerned at the physical examination. The blood pressure in this case was 230/125 and it seemed reasonable to assume the presence of a Goldblatt mechanism, caused by the shunt (obviously a pre-formed condition). The case has now been operated upon and it turned out that removal of the kidney did not cure the hypertension, which turned out to be caused by a septemic lupus erythematosus.

In brief summary, out of 62 instances of arterial hypertension subjected to renal arteriography an involvement of the renal artery was observed in 5 males and 5 females. In only one case, a man aged 55, was an operation by means of a patch graft deemed justified, so far with scanty results. In another man, aged 26, removal of the hypoplastic kidney was followed by recovery. In still another man removal of a renal tumor (representing a shunt) was followed by recovery.

Instances with fibromuscular hypoplasia have not been included in this study since they are dealt with in another paper, by Björk and Fagerberg (8 b). The present material from this clinic of such cases is confined to six instances.

Comment and discussion

When evaluating the present observations it should be borne in mind that they are derived from a selected sample of patients.

However, roentgenological evidence of interference with the arterial blood supply was found in only a minority of instances, in 10 cases out of 62, approximately the same incidence as that encountered for renal hypoplasia. The clinical details in most instances of renal arterial stenosis made it rather unlikely that the presence of such a stenosis could be of any but additional importance, at least as far as this material was concerned. It should also be remembered that the investigation recently carried out by Schwartz and White (18) seems to

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Clinical Evaluation of a Rapid Enzyme Strip Method (Dextrostix) for Blood Glucose Estimation[1]

By

BENGT SCHERSTÉN

There is an increasing demand for quick and cheap screening tests for mass surveys as well as for use in the general practitioner s surgery They may also prove useful in small hospitals without modern laboratory facilities

The Ames Company recently devised a method for determining the blood sugar with a paper strip Dextrostix Rennie et al (5) compared this technique with the Hoffman ferricyanide method They found that Dextrostix tended to overestimate low glucose concentrations and to underestimate high concentrations The variation of the values, irrespective of the sugar level was ascribed to variation in the volume of blood applied to the strip Cohen et al (2) compared the values obtained with a ferricyanide autoanalyzer method for determinations of blood sugar in venous blood with the manual glucose oxidase method described by Marks (4) for capillary blood and with Dextrostix They found good agreement over the entire range of values 40 to 200 mg per 100 ml, obtained with all three methods

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This paper is concerned with a detailed comparison between the glucose oxidase method performed on capillary blood and the Dextrostix technique

Methods and materials

The test strip Dextrostix (Ames Co Stoke Poges England) is made of stiff absorbent cellulose The active end is impregnated with a buffered mixture of glucose oxidase peroxidase and a chromogen system, and is coated with a semipermeable membrane to prevent staining by red blood-cells

As recommended by the manufacturers a large drop of capillary blood is quickly spread on the active end of the strip After exactly 60 seconds the drop is washed off by a jet of cold water from a plastic bottle The colour of the active end is then immediately compared with the colour chart printed on the label of the bottle

The scale of colours ranges from pale grey at 40 mg of glucose per 100 ml blood through deepening shades of blue grey at 65 90 130 150 and 200 mg According to the manufacturer s instructions the blood sugar

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TABLE I Determination of glucose by glucose oxidase method according to Marks (4)

The random error of the method

Range of blood concentrations (mg/100 ml blood)	Random error of the method (mg/100 ml blood)	Random error of the method (%)	Number of duplicate determinations
50–99	±1.8	±2.35	31
100–149	±2.0	±1.60	27
150–250	±4.0	±2.23	25
Mean		±2.1	
Total			83

TABLE II Comparison of different methods of applying the drop of blood on the active end of the strip

		Strip estimate	
Patient	Glucose oxidase method (mg/100 ml blood)	Blood applied by a pipette (mg/100 ml)	Blood applied directly (mg/100 ml)
A	70	65	90–130
		60	90
		65–90	65
B	83	90	65
		90	130
		90	130
C	172	150	130
		150	150
		150	150–200
D	220	200	130–150
		200	200
		200	150
E	115	90	90
		90 130	130
		90 130	130–150

Mean of three determinations

a pipette to the active end of the strip were compared with these obtained with the pipette method. The results were checked by the glucose oxidase method. Strips were treated and read in random order, the examiner being unaware of the method by which the blood had been applied (table II). The readings tended to vary more widely when the blood had been applied

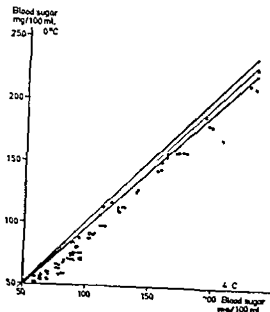


Fig 1 Correlation between blood sugar values (glucose oxidase method) in the same sample determined after storage at 20 C and at 4°C for three and a half hours The line of perfect correlation is flanked by lines indicating the random error of glucose oxidase method

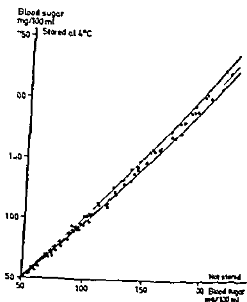


Fig 2 Correlation between blood sugar values (glucose oxidase method) in the same sample determined immediately and after storage for three and a half hours at 4°C. The line of perfect correlation is flanked by lines indicating the random error of the glucose oxidase method

values given for the first five hues are mid-hue values The hue marked 200 mg per 100 ml indicates a blood sugar of 200 mg or more The manufacturers also advise the use of interpolated values for colour readings falling between the colours of the chart

Blood was obtained from persons in whom field screening (1) with Clinistix (Ames Co) had given positive results Capillary blood was collected simultaneously for the Dextrostix test and for duplicate glucose oxidase determinations Ten samples were obtained from each patient during a three-hour oral glucose tolerance test carried out in the way described elsewhere (1) Fifty glucose tolerance tests were performed with altogether 490 glucose determinations

The Dextrostix method

The ear lobe or fingertip was pricked and a drop of blood was collected and applied to the active end of the strip with a capillary pipette

The glucose oxidase method

The method described by Marks (4) was slightly modified regarding the volume of the reagents to enable the use of automatic pipettes In the technique employed the blood samples were collected during the three hours of the glucose tolerance test and then analyzed simultaneously The slow but significant disappearance of glucose (4) unless the blood proteins were precipitated and separated as soon as possible from the protein free fluid could practically be prevented by keeping all samples at + 4 C (figs 1 and 2)

The random error (3) of the glucose oxidase method as judged by duplicate tests was ± 2 1 per cent (table I)

Results

Application of blood on the strip

In a separate series the results obtained when blood from the fingertip or ear lobe was applied directly, i e, without

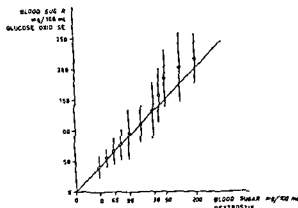


Fig 3 Comparative capillary blood sugar determinations Dextrostix strip readings against glucose oxidase method Mean ± 2 S D

directly The variation was probably due to inaccurate timing which was found to be of crucial importance The thickness of the blood film also tended to be uneven with the result that it was difficult to match with a single hue of the chart

The results were the same whether read in daylight by a window or in artificial light (table III) The actual thickness of the blood film proved less important than unevenness of the thickness of one and the same film (table III)

Difference between observers

A series of samples was read by four untrained and two trained examiners and the results were compared The results were checked by the glucose oxidase method Strips were treated and

TABLE V Comparison between Dextrostix and glucose oxidase method

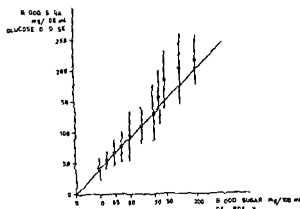
Strip estimates (mg/100 ml blood)	Glucose oxidase estimates (mg/100 ml blood)		No of tests
	Mean ± 2 S D	Range	
40	41 ± 18	– 59	11
40 65	56 ± 15	41 – 71	36
65	67 ± 22	45 – 89	61
65 90	77 ± 25	52 – 102	65
90	94 ± 40	54 134	25
90 130	110 ± 31	79 – 141	34
130	132 ± 64	86 – 178	[illegible]0
130 150	157 ± 44	113 – 201	84
150	184 ± 46	138 – 230	43
150 200	202 ± 57	145 – 259	23
200	215 ± 40	17[illegible] –	[illegible]8

TABLE III Comparison of different methods of applying the drop of blood on the active end of the strip and effect of lighting conditions

Patient	Strip estimate (mg/100 ml)		Glucose oxidase method (mg/100 ml)	Source of light (mg/100 ml)	
	Thin	Thick		Daylight	Artificial
A	65	65	70	65	65
	65	65		65	65
	65	65–90		65–90	65–90
B	90	90	83	90	90
	65–90	90		65–90	65–90
	90	90		90	90
C	130–150	150	172	150	150
	150	150		150	130–150
	130–150	150		150	150
D	200	200	220	200	200
	200	200		200	200
	200	200		200	200
E	90–130	90	115	90	90
	90	90–130		90–130	90–130
	130	90–130		90–110	90

TABLE IV Comparison of Dextrostix readings made by different observers

True value (mg/100 ml)	Readings by 4 untrained observers (mg/100 ml)						Readings by 2 trained observers (mg/100 ml)					
	40	65	90	130	150	200	40	65	90	130	150	200
82		3		1				2				
83	1	3						2				
91		4						2				
95		3		1					2			
119		1	1	2					2			
153			2	2						1	1	
155			1	2	1					1	1	
162			2	1	1					1	1	
183				2	2					1	1	
218					2	2					1	1
224					4							2
257					2	2						2
314					2	2						2



F g 3 Comparative cap llary blood sugar determ nat ons Dextrost x s np read ngs against glu ose oxidase method Mean ± 2 S D

directly The variation was probably due to inaccurate timing which was found to be of crucial importance The thickness of the blood film also tended to be uneven with the result that it was difficult to match with a single hue of the chart

The results were the same whether read in daylight by a window or in artificial light (table III) The actual thickness of the blood film proved less important than unevenness of the thick ness of one and the same film (table III)

Difference between observers

A series of samples was read by four untrained and two trained examiners and the results were compared The results were checked by the glucose oxidase method Strips were treated and

TABLE V Compar son betwe n Dextrost x and glucose ox dase method

Str p est mates mg 100 ml blood	Glucose oxidase estimates (mg/100 ml blood) Mean ± 2 S D	Range	No of tests
40	41 18	59	11
40 65	6 ± 15	41 71	36
65	67 22	45 89	61
65 90	77 ± 25	52 102	65
90	94 ± 40	54 134	25
90 130	110 ± 31	9 141	34
130	132 + 64	86 1 8	50
130 150	157 + 44	113 201	84
150	184 ± 46	138 230	43
150 200	202 57	145 259	23
200	215 40	175	58

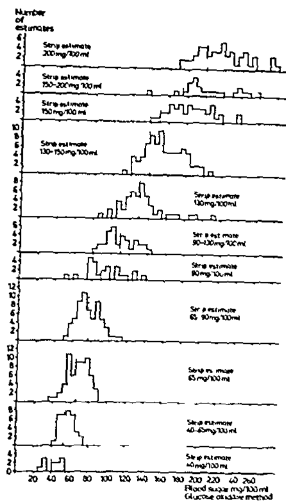


Fig 4 Frequency distribution of the blood sugar values according to glucose oxidase method at the different levels of strip estimates.

read in random order (table IV) The untrained group tended to report lower values, especially when the glucose concentrations were high

Comparison between Dextrostix and glucose oxidase method

It is clear from fig 3 and table V that in 40 to 130 mg glucose per 100 ml blood both methods gave almost identical re sults Between 130 and 200 mg glucose per 100 ml blood the strip method tended to give somewhat lower values The statement made by the manufactures

TABLE VI Evaluation of Dextrostix as a means of separating capillary blood sugar values above and below 120 mg/100 ml blood

Screening Dextrostix	True value >120	<120	Total
Positive	243	15	258
Negative	12	220	232
Total	255	235	490

TABLE VII Evaluation of Dextrostix as a means of separating capillary blood sugar values above and below 200 mg/100 ml blood

Screening Dextrostix	True value >200	<200	Total
Positive	39	19	58
Negative	24	408	432
Total	63	427	490

that the values given on the label represent mid line values could only be confirmed for estimates between 40—130 mg glucose per 100 ml blood (fig 4)

It was considered of interest to test the accuracy of the Dextrostix technique at the levels 120 mg per 100 ml blood and at 200 mg per 100 ml blood

The 120 mg glucose per 100 ml blood two hours after an oral glucose tolerance test, is often taken as the upper limit of the normal range of variation Two hundred mg glucose per 100 ml blood may be taken as a sign of unsatisfactorily controlled diabetes

It appears from fig 4 and table VI that Dextrostix gave false negative values in 12 out of 255 cases (5 per cent) and false positive values in 15 out of 235 cases (6 per cent) when screening at the level of 120 mg glucose per 100 ml blood At the > 200 mg level (table VII) the value was fictitiously low in as many as 24 out of 63 cases (38 per cent) Below 200 mg glucose per 100 ml blood the Dextrostix value was correct in 408 out of 427 instances (96 per cent)

In an attempt to improve the test the effect of excluding the 150 mg hue was studied A new series of 197 tests was made No interpolated values were used This improves the accuracy above 200 mg glucose per 100 ml blood (table VIII) In only 5 per cent of the cases with a true value above 200 mg glucose per 100 ml blood did the method give fictitiously low results This improvement was however achieved at the expense of the number (12 per cent) of false positive values in patients with a blood sugar level below 200 mg per 100 ml The price for the improvement in the accuracy of the method for the high levels was considered acceptable

TABLE VIII Evaluation of Dextrostix as a means of separating capillary blood sugar values above and below 200 mg/100 ml blood The 150 mg hue is ignored

Screening Dextrostix	True value		
	>200	<200	Total
Positive	35	20	55
Negative	2	140	142
Total	37	160	197

Summary and conclusions

Evaluation of a paper strip method for quick blood glucose determination Dextrostix (Ames Co) appeared to justify the following conclusions

The accurate timing of 60 seconds is of crucial importance

The blood drop should be applied to the active end of the strip by means of a pipette

The actual thickness of the blood film is of less importance provided it is even

The results are the same whether the strips are read in daylight or artificial light

Untrained observers are inclined to underestimate the strip value especially at higher blood glucose concentrations

The method has a tendency to under estimate high glucose concentrations, especially at the levels of 130 to 200 mg per 100 ml blood

The accuracy of the results appears to depend upon the correctness of the colour chart on the label of the bottle The 150 mg per 100 ml hue on the Dextrostix colour chart appears to be incorrect

A factor contributing to the variability of the estimates of glucose concentrations of especially interesting levels is probably the wide range at each hue block In spite of these wide ranges the method seems to discriminate glucose values from below 40 up to 130 mg per 100 ml blood and below and above 200 mg per 100 ml blood More reliable results can

be obtained in the level of 200 mg glucose per 100 ml blood if the 150 mg hue is ignored

If the limitations of the method are borne in mind, the method may be considered as a useful screening procedure in diagnosing hypoglycæmia or hyperglycæmia A strip reading indicating 130 mg glucose per 100 ml blood (range 86—178) or less may indicate a satisfactory blood sugar level in diabetics In combination with the recording of glycosuria by the patients themselves Dextrostix may prove a useful adjunct in the management of diabetics Even in smaller hospitals without modern laboratory facilities the test may be useful The results also suggest that the test may be used as screening test in population surveys

Acknowledgement

The test strip Dextrostix was kindly supplied by AB Meda Gothenburg Sweden

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From Oslo Sanitetsforenings Revmatismesykehus (Head E Kås M D), Oslo Norway

Polymyalgia Rheumatica

and its Relation to Arteritis Temporalis

By

O A Kogstad

Polymyalgia rheumatica (5 10) also known as anarthritic rheumatoid disease (2 3 4) and pseudopolyarthrite rhizomelique (9) — can be recognized as a characteristic clinical entity among the rheumatic diseases It occurs predominantly in the older age groups and women are affected twice as often as men The initial symptoms are usually generalized malaise fatigue fever and loss of weight Myalgias the dominating symptom is usually localized to the neck and shoulder muscles although the pelvic girdle and other muscles may be involved Permanent clinical or radiological signs of arthritis in related joints are not evident The erythrocyte sedimentation rate is elevated and a hypochromic anemia is often found Prognosis is usually good with the duration of symptoms lasting from under a year to a few years Corticosteroids produce a striking symptomatic improvement in the management of these patients but do not appear to influence the duration of the disease

The diagnosis may be difficult, and requires a period of observation and a careful exclusion of other diagnoses The cause of the syndrome is not known A relationship between polymyalgia rheumatica and temporal arteritis has been postulated by several authors (1, 7 12, 13 14 15, 16)

In the following report, a study of the occurrence of temporal arteritis in patients with polymyalgia rheumatica is presented Obvious similarities suggest that they may be reactions of the same nature, differing only in localization

Material

Seventy cases of polymyalgia rheumatica 46 women and 24 men with age of onset from 50 to 80 years have been examined and followed up in the two-year period 1962—1964 at Oslo Sanitetsforenings Revmatismesykehus This hospital is annually admitting about 1 000 patients with rheumatic diseases from all parts of Norway Ten of the cases were out patients No trends of special geographic location of the disease were

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TABLE I Symptoms and signs

	Polymyalgia rheumatica		
	With temporal arteritis	Without temporal arteritis	Total
No of cases	13 (7♀ − 6♂)	57 (35♀ − 18♂)	70 (46♀ − 24♂)
History of			
rheumatic fever	2	4	6
erythema nodosum	0	5	5
rheumatoid arthritis in family	1	6	7
General malaise			
fatigue	13	57	70
fever	9	38	47
loss of appetite and weight	11	53	64
Myalgias			
shoulder girdle	12	52	64
pelvic girdle	13	51	64
other locations	8	29	37
Muscle tenderness	10	54	64
Muscle stiffness with limited shoulder joint movements	9	51	60
Pain localized to joints or periarticular tissue	3	53	56
Transient hydrops			
of the knee joint	1	6	7
of the shoulder joint	0	1	1
of the sternoclavicular joint	1	2	3
Joint radiography osteoarthritis	7	29	36
Padding in the supraclavicular region	1	6	7

found In the series 13 patients had symptoms of temporal arteritis, confirmed by biopsy in 12 cases

Symptoms (see also table I)

The time of onset was distributed evenly throughout the year Of possible precipitating factors, 10 patients initially had upper respiratory infections The myalgia was mainly acute in onset and localized to the muscles of the shoulder and pelvic girdle Quite frequently general malaise, fatigue fever and weight loss were found in the initial stage of the disease The symptoms sometimes developed more gradually and with varying degrees of severity Muscle stiffness accompanied by limitation of joint movement, especially in the

shoulders, was a characteristic finding A slight degree of muscle tenderness was usually present In patients with severe symptoms with weight loss and long periods of inactivity muscular atrophy was seen In some patients pain was localized to the joints, but no definite signs of arthritis were found and X ray examination showed only degenerative changes of the osteoarthritic type Seven patients not on corticosteroids had diffuse swellings in the fossae supraclaviculares This symptom has been mentioned by only one previous author (7) The "padding' had a rather lax consistency, and no swelling of the sternoclavicular — or acromioclavicular joints could be found (fig 1)

Muscle biopsies from shoulder and calf muscles in 3 patients were all negative



Fig 1 "Padding in the supraclavicular region in a patient with polymyalgia rheumatica *before corticosteroids were given*

Laboratory data (see also table II)

The erythrocyte sedimentation rates were markedly elevated the upper levels being between 43 and 131 mm/hour Most of the

TABLE II Laboratory findings

		Polymyalgia rheumatica		
		With temporal arteritis	Without temporal arteritis	Total
No of cases		13	57	70
E S R (highest) mm hr	Range	44 – 129	43 – 131	43 – 131
	Mean	91 1	83 8	84 9
Hemoglobin (lowest)	Range	75 – 85	63 – 107	63 – 107
	Mean	76 6	82 9	82
Plasma fibrinogen (g/100 ml)	Range	0 40 – 0 86	0 30 – 0 53	0 30 – 1 53
	Mean	0 56	0 62	0 61
Plasma electrophoresis				
Low albumin		7	26	33
Raised α globulins		1	1	2
Raised $α_2$ globulins		10	32	42
Raised β globulins		0	0	0
Raised γ globulins		4	6	10

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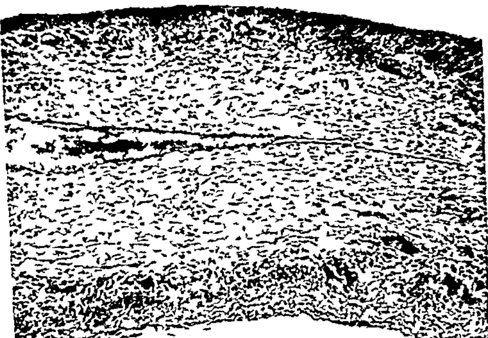


Fig 2 Biopsy from the temporal artery of a patient *9 1/2 months after onset of polymyalgia* rheumatica symptoms Temporal headache and local signs of arteritis then developed together with a flare up of the general symptoms Longitudinal section of the artery shows great proliferation of connective tissue in the intima resulting in a tiny lumen In the inner layer of the media several multinucleate giant cells are seen together with a cellular infiltrate composed mainly of lymphocytes (Int magn 64

Course of disease

The patients were followed up at intervals of less than 3 months mostly 2—4 weeks In addition to a clinical examination the following laboratory investigations were repeated ESR hemoglobin rheumatoid factor antistreptolysin titer plasmafibrinogen electrophoresis white cell count and X ray studies of any joints with pain hydrops or limited joint movements The observation time varied from 10 to 28 months 18 patients became symptom free within 7 28 mean 18 months after the onset of symptoms and have stopped using drugs 23 patients are almost symptom free 27 have improved and 2 patients have little change in their symptoms 30 patients are still on a low daily steroid dose (2 5—7 5 mg prednizone the maximum observation time being 23 months

Occurrence of temporal arteritis

Thirteen of the patients with polymyalgia rheumatica had temporal arteritis Biopsies from 12 of them showed the typical picture of "giant cell arteritis (fig 2) One patient refused biopsy but had obvious signs of arteritis with redness tenderness and infiltration localized to

patients also had a hypochromic anemia. Plasmafibrinogen was raised in the majority of patients, with a mean value of 0.61 g/100 ml. A control group of 10 patients with rheumatoid arthritis and with corresponding elevation of E.S.R. also had higher plasmafibrinogen levels than normal (mean 0.60 g/100 ml). Electrophoresis of plasma proteins usually was pathological, but the findings were not specific. The rheumatoid factor was found in only one of the 70 patients 8 months after onset of symptoms. This patient was then readmitted with symptoms of rheumatoid arthritis, and a positive RA test was found on examination. No LE cells were found on the blood films from 65 patients and antistreptolysin titers were within normal values in 48 patients examined.

Differential diagnosis

Polymyalgia rheumatica may have an initial course similar to rheumatoid arthritis. However, the Waaler-Rose tests have always been negative in our series except for the one patient who developed rheumatoid arthritis. Six of our patients had transient joint swelling, but X-ray examination only showed osteoarthritic changes. Hydrops subsided within 1—4 weeks and no further signs of rheumatoid arthritis developed on follow-up examinations. Polymyalgia rheumatica and disseminated lupus erythematosus (LED) may have similar systemic reactions but neither LE-cells nor other signs of LED were found in our series. In dermatomyositis or polymyositis muscular atrophy and weakness are more conspicuous than myalgias. Creatine excretion, serum transaminase, electromyography and muscle biopsies can establish the diagnoses.

The differential diagnosis against polyarteritis nodosa with typical visceral and neurological symptoms is usually no problem. The vascular lesions may sometimes be difficult to differentiate histologically from giant cell arteritis, and they are described together in one patient (15). Certain virus infections may initially run a course similar to polymyalgia rheumatica with predominantly general symptoms. Patients with fibrositis localized to the shoulder girdle muscles lack general symptoms and have normal laboratory findings. Myeloma patients may have muscle pain and high ESR, but can be ruled out by urine analysis, electrophoresis, X-ray and bone marrow biopsy. Malignant tumors may be suspected where elderly people run a prolonged course of disease with diffuse pain, weight loss, anemia and high ESR. In many patients with polymyalgia rheumatica it will therefore be necessary to exclude malignant diseases.

Treatment

Salicylates were given initially to the majority of the patients and 9 of them improved satisfactorily. 51 patients with severe symptoms were given corticosteroids with striking improvement. Corticosteroids sufficient to suppress the symptoms were also given to all patients with temporal arteritis. The initial dose was usually 20 mg prednisone daily, with a gradual reduction to a maintenance dose in the range 5—10 mg daily. Ten patients were given phenylbutazone (Butazolidin 0.2 g daily) alone and 27 patients were given phenylbutazone during withdrawal of steroids, with good results. In addition physiotherapy was given in all cases.

and that an arteritis may be the underlying pathogenic origin. Polasky et al (14) reported giant cell arteritis, presenting itself clinically as polymyalgia rheumatica in an 82 year old female. The histological diagnosis was established by examination of the arteries of the uterus. We have muscle biopsy studies in only 3 patients but in all without pathological findings. Most authors are also reporting negative muscle biopsies.

Gordon et al. (11) made histological studies from the shoulder and deltoid muscle in 6 cases of polymyalgia rheumatica and found non specific inflammatory changes.

Bagratuni (2, 3, 4) is of the opinion that polymyalgia rheumatica is a variant, or a benign form, of rheumatoid arthritis. The relatively high frequency of rheumatoid arthritis among members of the the same families in our series, and the high incidence of rheumatic diseases in the history of our patients, may perhaps indicate that there is some relationship between these diseases. A small minority of patients with the syndrome of polymyalgia rheumatica are also reported to develop rheumatoid arthritis (4, 11, 16).

Summary

Seventy patients with polymyalgia rheumatica were observed by the author during the two year period 1962—1964. The observation time varied from 10 to 28 months. 13 patients also had signs of temporal (giant cell) arteritis, confirmed by biopsy in 12. Local signs were absent in 2 of these cases with positive biopsies but the patients had temporal headache. In 9 of the 13 patients with temporal arteritis, symptoms of polymyalgia preceded those of arteritis by 5 months to 2 years. In the other 4, symptoms of arteritis appeared first. Steroid therapy was used in 51 patients with severe symptoms with striking improvement. Two patients on steroid therapy subsequently developed temporal arteritis. Ocular complications were not observed. During the follow up 18 cases became symptom free within 7—28 (mean 16) months and have stopped using drugs.

No definite differences either in symptoms or in clinical or laboratory findings were found, whether or not the polymyalgia rheumatica was complicated by temporal arteritis.

The author discusses the connection between temporal arteritis and polymyalgia rheumatica.

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the temporal arteries All 13 patients had temporal headache and 4 of them also complained of pain radiating to the ear and to the side of the neck Two of the patients with positive biopsies did not show local signs of arteritis In further 4 patients with temporal headache, biopsies were done without pathological findings

Only 3 patients in this group had blurred vision All patients with verified temporal arteritis were examined by an ophthalmologist No signs of ocular involvement was found during an observation period of from 9 to 24 months

Two patients had signs of vasculitis in other locations, one from arteria carotis externa and one from arteria tibialis posterior

In 9 of the 13 patients with verified temporal arteritis, symptoms of polymyalgia preceded those of arteritis by 5 to 24 months (mean 12 months) In the remaining 4 patients the arteritis appeared first Two patients on corticosteroids (2 5 and 5 mg prednisone daily) subsequently developed temporal arteritis 8 and 9 1/2 months after onset of the polymyalgia-symptoms Both were at the time almost symptom-free, but had ESR values higher than 20 mm/hr

Comments (see table I and II)

Comparison of the patients with and without temporal arteritis shows 6 men and 7 women in the group with temporal arteritis, whereas in the other group there were 35 women and 18 men Arthralgias occurred more frequently in the group with temporal arteritis There were small, but not significant, differences in the laboratory findings No definite difference in symptoms, clinical signs or laboratory findings were found in patients with temporal arteritis as compared to the remaining cases The response to treatment was also similar, as was the course of the disease, although the average duration in patients with temporal arteritis was somewhat longer The arteritis in the temporal artery was an episode of shorter or longer duration, and it was accompanied by an exacerbation of the general symptoms of varying intensity Whether this is called a complication of polymyalgia rheumatica, a "rheumatoid form" of temporal arteritis, or a mixed form, is essentially of theoretical interest The practical consequence seems to be that all patients with polymyalgia rheumatica should be treated with corticosteroids as potential arteritis-temporalis patients, since steroids are thought to reduce the frequency and severity of ocular involvement (6) In our series we did not have any complications

Discussion

The high incidence of temporal arteritis namely 18 %, in our patients with polymyalgia rheumatica supports the theory that both syndromes represent reactions to the same pathogenic factor The individual mode of reaction to this factor may in some patients give polymyalgia rheumatica in others temporal arteritis or a combination of both

Paulley and Hughes (13) suggest that polymyalgia rheumatica is a variant of giant cell arteritis (temporal arteritis),

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From the Medical Clinic B (Renal Clinic) the Laboratory of Clinical Neurophysiology, University of Lund Lund Sweden, and the Paediatric Department University Hospital, Helsinki Finland

Ethylene-glycol Poisoning Treated by Haemodialysis

By

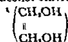
Karl Erik Hagstam David H Ingvar, Marianne Paatela and Hans Tallqvist

Severe poisoning and deaths after intake of ethylene glycol in man were described for the first time in 1930 (6 17, 37) During the 1940s and 50s several reports of similar cases of poisoning were published The mortality was throughout very high (41) The relevant literature and the current principles of treatment were reviewed by Friedman et al in 1962 (12) Haemodialysis for the purpose of removing the toxic substance and possibly its metabolites has been tried in a few cases (11 12 41) It has also been used in the treatment of acute renal failure secondary to ethylene glycol poisoning 11 12 27 29 41) A short account will be given here of the metabolism and toxicity of ethylene glycol A case of ethylene glycol poisoning with severe toxic manifestations referable to the brain and the kidneys will then be presented The cerebral disorder was studied by repeated electroencephalographic recordings The patient was dialysed three times He recovered

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Toxicological remarks

The bivalent alcohol ethylene glycol



is *extensively* used in the chemical industry and as anti freeze agent After ingestion some ethylene glycol is excreted unchanged in the urine both in man (16 18 19) and in animals (31) Oxalic acid in the urine has also been noted after ingestion of ethylene glycol in animals as well as in man (? 13 35) The amount of oxalic acid excreted in the urine varies from one animal species to another (13) It seldom equals more than 2 % of the ingested amount of ethylene glycol (14) Isotope studies in animals *in*dicate that the chief metabolite of the substance is carbon dioxide formed by further oxidation of one of the intermediary metabolites (possibly glyoxylic acid) in the oxidation chain whereby ethylene glycol is converted into oxalic acid (13 14) Observations made in animals suggest that ethanol can inhibit the enzymic breakdown of ethylene glycol (33) The metabolism of ethylene glycol in man has been studied only to a small *extent* and there is no certain evidence for the process outlined above

Experimental toxicity studies have been made by several authors Great *variations*

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The diagnosis was doubtful in this phase Poisoning was suspected but conclusive evidence was as yet lacking The subsequent course was characterized by severe acidosis and diminishing wakefulness Muscle reflexes disappeared but the patient s reaction to pain was preserved During administration of sodium bicarbonate and sodium lactate solutions the degree of wakefulness increased and in the evening of day 2 he answered when spoken to Urinary output decreased, despite administration of about 1 5 litres of fluid per 24 hours On day 3 B U N was 54 mg per 100 ml The degree of wakefulness diminished again He became delirious and had hallucinations Severe E E G abnormalities were observed (see special summary) On day 4 B U N was 89 mg per 100 ml and urinary output minimal Because of the renal failure he was transferred to the Medical Clinic B (Renal Clinic) at Lund on day 4

By then the diagnosis of ethylene glycol poisoning had been established Chemical analysis had shown that the bottle from which the boy had drunk contained a water solution of ethylene glycol (Department of Forensic Chemistry Institute of Forensic Medicine Helsinki The concentration was not determined Methanol could not be demonstrated

On admission to the Renal Clinic in the evening of day 4 the predominating clinical features were marked psychic and motor restlessness disorientation hallucinations and aggressiveness Slight declivous oedema and slight facial oedema were noted Respiration was regular and fairly deep at a rate of about 20 per minute Circulation was satisfactory Blood pressure was 150/80 mm Hg and pulse rate 120 per minute The pupils were slightly dilated of equal size and reaction to light was normal Muscle reflexes were brisk There was no paresis At admission the haemoglobin concentration was 11 7 g per 100 ml red-cell count 3 900 000 per mm white-cell count 11 500 per mm of blood Differential count neutrophils 87 eosinophils 1 % basophils 0 % lymphocytes 7 % monocytes 5 % Thrombocyte count 133 000 per mm of blood The serum-concentration of sodium was 154 mEq of potassium 5 9 mEq of calcium 4 6 mEq, of chlorides 110 mEq per litre of phosphate 10 2 mg per 100 ml of standard bicarbonate 16 5 mEq per litre and of albumin 8 6 g per 100 ml Non protein nitrogen (N P N) in the blood was 168 mg per 100 ml Proteinuria and microscopical haematuria were noted There were no oxalate crystals in the urine X ray of the chest showed no evidence of fluid retention Plain X ray of the abdomen showed enlarged kidneys measuring 11 5 × 6 5 cm on the right side and 11 5 × 6 cm on the left

Thus this was a case of almost total anuria in association with an acute psychotic condition not unlike the findings in some patients with uraemia and salt and fluid retention (2) Treatment with the artifical kidney (the Alwall type) was started within 4 hours of admission to the Renal Clinic The purpose was to try to eliminate any remaining ethylene glycol or metabolites thereof and to lessen the uraemic intoxication and the fluid retention The patient s violent restlessness made the treatment difficult and necessitated sedation with barbiturates and klormetiazol (Heminevrin) Slight muscle twitchings were noted on several occasions but he had no manifest convulsive attack. The first treatment with the artificial kidney lasted for 6 hours During this period the patient became calmer and mentally clear The serum-electrolyte levels became normal and N P N in the blood fell to 94 mg per 100 ml His body weight was reduced by 1 1 kg (4 %) No determination was made of ethylene glycol or its metabolites

On days 5 and 6 the patient was drowsy but answered adequately when spoken to Eye examination showed reduction of vision to 0 7 on the right and 0 5 on the left eye without correction The result was judged as somewhat uncertain in view of the patient s poor cooperation Slight retinal oedema was noted He was anuric and on day 7 a second dialysis treatment was performed on the indication of uraemia By then there was no clinical or laboratory evidence of over hydration During the dialysis, which lasted for 5 1/2 hours the degree of wakefulness

in toxicity between different animal species were noted (28, 32 and others). In man, the toxicity can be assessed mainly on the basis of clinical observations. The smallest lethal dose is stated to be around 100 ml (20). But there are reports of deaths after ingestion of up to 60 ml (43) and of survival after 240 ml (22).

In man, ingestion of ethylene glycol produces within a few hours nausea and sometimes vomiting of blood stained matter. Symptoms referable to the central nervous system, at first resembling alcohol intoxication such as hallucinations, stupor, coma and convulsions, appear within 12 hours. Besides toxic cerebral damage, hypocalcaemia has been proposed as a possible cause of the latter symptoms, but this has been confirmed in only a few cases (4). In cases where death occurred in this early phase, examination of the central nervous system showed unspecific changes in the form of oedema, capillary stasis, petechiae, degenerative nerve cell changes and signs of slight inflammatory processes (1, 12 and others), the kidneys contained oxalate crystals but showed generally no evidence of cellular damage (1, 12, 36, 42 and others). In survivors severe metabolic acidosis and not seldom signs of circulatory and respiratory failure and occasionally pulmonary oedema develop within 12—24 hours. The condition is often complicated by acute renal failure which within 3—4 days will be the increasingly predominating clinical feature. Microscopical examination of renal tissue in this phase shows in addition to the oxalate crystals mentioned above evidence of tubular necrosis of varying degree of severity together with slight glomerular changes manifest as increased numbers of cells and a slightly thickened basal membrane (e.g. 5). In lethal cases, evidence of myositis has been found in both cardiac and skeletal muscle (12).

Case report

On July 22, 1961 (day 1) a 10-year-old previously healthy Finnish boy while playing in a garage drank 100—150 ml of a clear colourless liquid kept in a brandy bottle. A few hours later he complained of a headache which was relieved by one tablet of acetyl salicylic acid. He fell asleep but woke up after 4 hours, showing symptoms of intoxication with an atactic gait, co-ordination difficulties, and mental confusion. He was admitted to the Paediatric Department at the University Hospital in Helsinki. During the ambulance journey, which took about 1 hour, he began to have generalized convulsions and became unconscious. On admission to the hospital, about 10 a.m., he was soporose. Muscle tension and muscle reflexes were normal. He had no neck stiffness and Babinski's phenomenon was not present. His eye grounds were normal. He had normal respiration of about 20 per minute. His heart and lungs were normal on physical examination. His pulse-rate was 80 beats per minute, blood pressure 120/90 mm Hg. There were no signs of corrosion in his mouth or throat. His abdomen was normal on palpation.

In connection with blood sampling he had convulsions which were interpreted as tetanic spasms and for which he was given calcium gluconate and 50 % glucose solution intravenously and pentobarbital intramuscularly. Haemoglobin concentration in the blood was 15.8 g per 100 ml, the red cell count 5 900 000 per mm³, haematocrit 47 % and the leucocyte count 36 000 per mm³. Differential count: neutrophils 85 %, eosinophils and basophils 0 %, lymphocytes 11 %, and monocytes 4 %. In the serum the sodium level was 141 mEq, the potassium level 5.3 mEq, the calcium level 6.3 mEq (after administration of calcium chloride), chlorides 98 mEq and bicarbonates 8 mEq per litre. The blood sugar level was 0.14 g per 100 ml. Blood urea nitrogen (B.U.N.) was 17 mg per 100 ml. Proteinuria and microscopical haematuria were noted. The urine also contained great amounts of oxalate crystals. The cerebrospinal fluid was clear and colourless; there was no increase of cells. Nonne's and Pandy's tests were negative, albumin concentration was 30 mg per 100 ml and the sugar level 0.055 g per 100 ml. The pressure of the cerebrospinal fluid was not measured.

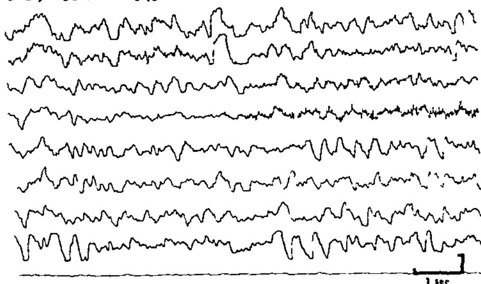


Fig 2 EEG sample from day 3 Monopolar recording from left and right frontal (1 2) temporal (3 4) parietal (5 6) and occipital (7 8) regions Calibration 100 μV

well as slight responsiveness to arousing stimuli There was still however a substantial amount of high voltage slow waves on both sides The same finding was made on day 18 (fig 3)

On day 26 when marked improvement of the patient's clinical condition had taken place the EEG was almost normal fig 3) Only a very small amount of some low voltage 4—7 cps activity was still seen No focal abnormalities were present On hyperventilation the slow wave content of the record increased markedly Two days later another EEG showed only a slight admixture of low voltage theta waves A control EEG examination 6 weeks following the intoxication still showed some slight general changes of unspecific type

Comments

The essential clinical features in the case described here were consistent with those generally seen in ethylene glycol poisoning The convulsions occurring initially in the course (day 1) were probably caused by the toxic cerebral effects of ethylene glycol or its metabolites possibly together with local oedema (5 8) An accentuation of the latter factor may have triggered the convulsions during the dialysis treatment on day 7 Rapid haemodialysis can produce a temporary osmolarity gradient between the blood and the cerebrospinal fluid and between the latter and the brain cells, with resulting increased pressure and cerebral oedema (9, 23 34 39, 40) Slower haemodialysis administration of substances which raise the extracellular

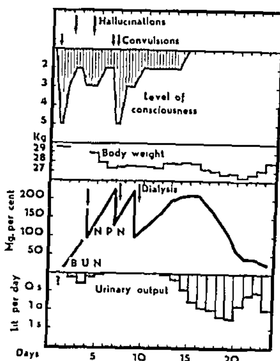


Fig 1 Diagram showing the course in the reported case hallucinations convulsions levels of consciousness (1 wide awake 2 somewhat drowsy but answers promptly 3 drowsy answers sluggishly, 4 reacts to speech but does not answer 5 reacts only to pain 6 no reaction to pain) body weight B U N and N P N dialysis treatments urinary output

diminished N P N fell from 224 to 132 mg per 100 ml, and the body weight was reduced by 0 2 kg Towards the end of the dialysis he had several attacks of severe generalized convulsions with unconsciousness which lasted for about 12 hours A tendency to blood pressure fall was offset with Metaraminol (Aramine)

On day 8 the patient responded when spoken to, though not always adequately His general condition was still poor Prednisolon (Precortalon®), 12 5 mg twice daily, was started, partly because of the tendency to shock, partly in the hope of reducing the tissue reaction in the brain and the kidneys (1, 5, 12) Antibiotics, gamma globulin and anabolic steroids were also given for prophylactic purposes

On day 9, a third dialysis treatment was carried out, and during the course of it N P N fell from 222 to 97 mg per 100 ml, the moderately raised potassium level returned to normal, and the body weight was reduced by 0 4 kg During the next few days his general condition improved gradually, as urinary output increased N P N rose to a maximum of 212 mg per 100 ml on day 14 and after another 6 days it was normal Eye examination on day 18 still showed reduced vision (right eye 0 5, left eye 0 4 without correction) Bilateral mydriasis and partial loss of accommodation were noted There was no retinal oedema The symptoms were interpreted as due to central nervous damage On day 24, eye examination showed no abnormalities On day 26, otoneurological examination was normal Endogenous creatinine clearance was 70 ml per minute, and serum creatinine 0 71 mg per 100 ml On day 28 the patient was sent back to the Paediatric Clinic in Helsinki Psychological testing by the method of Terman Merrill Lehtovaara showed an intelligence quotient of 137 At the time of the follow up examinations 2 and 3 months later, the patient was back at school and seemed to have recovered completely

Summary of the E E G recordings

The first E E G recording was made in Helsinki on day 3 (fig 2) It showed a highly abnormal pattern over both hemispheres with absence of alpha rhythm and a generalized slow wave pattern A renewed examination on day 5 revealed a further deterioration with a dominant activity of 0 5—3 cps (fig 3) It was also observed that the patient did not react to arousing stimuli and the E E G following such measures remained uninfluenced

On day 12 a slight regression of the E E G changes was noted There was some 6 cps activity in the background as

the clinical symptoms Besides the exogenous intoxication, the uraemia and possibly cerebral oedema may also have contributed to the E E G changes (7, 26 and others)

The fact that a severe generalized abnormality was present as early as day 3, when B U N was only 54 mg per 100 ml argues against uraemia as the sole cause of the changes Later in the course, however, uraemia can have been a causal factor of importance The return of the E E G to normal corresponds in time to the disappearance of the uraemia

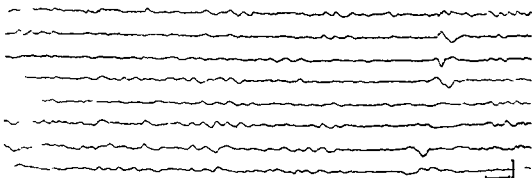
The value of dialysis treatment of renal failure secondary to ethylene-glycol poisoning is unquestionable But it has not yet been established beyond doubt whether the method is effective in removing the toxic substance or its metabolites (10) In view of the high mortality from such poisoning and the lack of other specific efficient methods of treatment we are of the opinion that haemodialysis should be used if the amount of ethylene glycol ingested by the patient approaches the lethal dose for man When the ingested amount is unknown dialysis should be considered if severe symptoms of poisoning appear early in the course

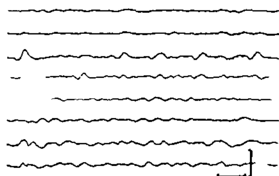
Summary

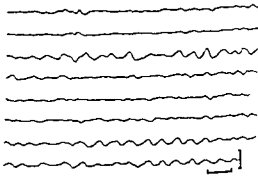
A short survey is given of the metabolism and toxicity of ethylene glycol A case of ethylene glycol poisoning in a 10-year-old boy is presented Severe toxic manifestations referable to the brain and the kidneys were noted The cerebral condition was studied by repeated E E G examinations The E E G abnormalities and their disappearance paralleled the clinical course Because of the exogenous intoxication, electrolyte fluid retention with suspected cerebral oedema and uraemia, a treatment with the artificial kidney was performed soon after admission to the Medical Clinic B (Renal Clinic) A marked clinical improvement was noted Two further dialysis treatments were given because of uraemia The patient recovered It is probable that dialysis treatment may be of benefit in cases of severe ethylene-glycol poisoning even in the absence of uraemia

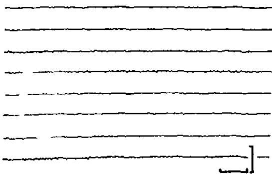
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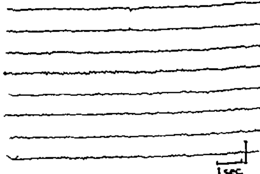


Fig 3 E E G samples during recovery Bipolar recordings from right and left frontal (1 5) fronto-temporal (2, 6), temporal (3, 7), and temporo-occipital (4 8) regions Calibration 300 μV

osmolarity, and possibly ultrafiltration, appear to reduce the risk of oedema of this kind (2, 24, 25, 34, 38, 40)

E E G changes in ethylene-glycol poisoning have been described earlier (12, 21) The severe generalized E E G changes in our case suggest a marked impairment of cerebral function The successive disappearance of the E E G abnormalities corresponded well with

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From the Medical Clinic (Head G Monasterio, M D), the University of Pisa, Pisa Italy

Clinical Applications of Quantitative Radiocardiography

II Results in Patients with Hypertensive and Coronary Heart Disease without Clinical Signs of Cardiac Decompensation[1]

By

Attilio Maseri and Luigi Donato

A passive increase of left atrial pressure resulting from ventricular failure leads to an augmentation of the distending pressure in the pulmonary circulation As a consequence an increase of pulmonary blood volume might be expected

The availability of a radiocardiographic method for the determination of circulating pulmonary blood volume in clinical routine (7—9) prompted us to study the behaviour of pulmonary blood volume in cardiovascular diseases leading to progressive hypertrophy and dilatation of the left ventricle

Our aim was to ascertain whether the simultaneous measurement of cardiac output and of pulmonary blood volume could supply information on the state of compensation of the left ventricle in the presence of hypertrophy and dilatation

Valvular heart diseases were purposely excluded from the study for sake of homogeneity

Submitted for publication May 17 1965

Technique

Instrumentation technique and experimental procedure have been described in detail elsewhere (7—9) and will be only briefly summarized

A photomultiplier (RLD 2 Tracerlab) with a NaJ (Tl) 1 5 × 1 5 inches crystal is located in a 20 mm thick lead cylindrical collimator 40 mm in diameter and recessed 80 mm from the external opening

To obtain radiocardiograms (RCG) the probe was positioned 1 cm away from the anterior chest wall over the 4th intercostal space on the left sternal border Fifty μC of radioiodinised serum human albumin (RISHA ^{131}) (Sorin—Saluggia (free iodine < 2 %)) diluted up to 0 2 ml with saline were flushed into the superior vena cava through a polyethylene catheter introduced percutaneously by 0 5 ml saline The tracing was recorded by a ratemeter on a dual direct writing recorder (Texas Instr Inc) together with an electrocardiographic lead Five minutes after the injection the net

[1] The data reported in this paper have been presented in a conference given by one of the authors (Donato) on March 9 1965 at the Swedish Medical Society in Stockholm

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AP = arterial pressure VP = venous pressure BV = blood volume HR = heart rate ci = cardiac blood volume

BV (ml/m²)	HR (cycles/min)	ci (l/min/m²)	SV (ml)	$\overline{PCT}$ (heart cycles)	PBV (ml)	PBV (ml/m²)	PBV/BV (%)
2 916	65	3 [illegible]	105	5 5	577	294	10 0
2 179	82	3 8	84	5 5	462	244	11 2
2 80	79	3 8	94	6 0	564	282	10 9
2 327	70	3 1	70	—	—	—	—
2 625	80	3 0	60	7 0	432	257	9 8
2 986	71	2 8	70	6 0	420	236	7 9
2 807	79	3 2	78	6 5	507	264	9 4
2 654	81	3 8	85	6 0	510	280	10 5
2 620	56	2 3	81	6 0	486	248	9 5
2,568	56	2 3	67	6 5	435	267	10 4
2 625 9	71 9	3 15	79 4	6.1	488 1	263 5	9 95
233 0	10 0	0 63	13 5	0 4	53 6	18 6	0 50

I The first group comprises 10 patients (3 females and 7 males) with no signs of left ventricular enlargement including 4 cases of HHD 2 cases of CCI and 4 cases with MI two of which are in the second stage

II The second group comprises 10 patients (3 females and 7 males) with slight left ventricular enlargement (+) 3 with HHD 3 with CCI 2 with CT and 2 with MI in the second stage

III The third group comprises 13 patients (2 females and 11 males) with enlarged left ventricle (++) or (+++) including 3 cases with HHD 2 with CCI and 8 with one or more MI

Results

The results obtained are reported in table I–III and illustrated in figs 1—3

Cardiac output was on average 3 15 l min m² in the first group 3 21 l/min m² in the second and 2 68 l/min/m² in the third group It was below 3 l/min/m² in 3 subjects in the first group (two aged 80 and 73 years respectively and one with MI in the second stage and hypotension), in 2 subjects in the second group (one with MI in the second stage and hypotension and one with CCI) and in 10 subjects in the third (fig 1)

Pulmonary circulation time $\overline{PCT}$ averaged in the first and second group respectively 6 11 and 6 05 heart cycles showing only slight variations (between 5 and 7 heart cycles) In the 3rd group it averaged 7 30 heart cycles, with values between 9 and 10 heart cycles in three cases (fig 1)

Pulmonary blood volume PBV in the three groups averaged respectively 263 5 ml/m² 264 6 ml/m² and 275 4 ml/m² in all instances being within the normal range It was below the average in patients with myocardial infarction in

TABLE I Group I Patients without signs of left ventricular enlargement on standard chest film index, SV = stroke volume, $\overline{PCT}$ = mean pulmonary circulation time, PBV = pulmonary

Case	Diagnosis	Sex	Age	BSA (m^2)	AP (mm Hg)	VP (cm H_2O)
012 P	Renal hypertension	M	59	1 96	240/125	–
015 P	Myocardial infarction 2nd stage	F	42	1 89	135/85	7
028 P	Essential hypertension	M	42	2 00	180/110	8
044 P	Chronic coronary insufficiency	F	53	1 58	150/90	6
052 P	Myocardial infarction 3rd stage	M	60	1 68	145/90	8
063 P	Myocardial infarction 2nd stage	F	59	1 78	105/80	–
077 P	Renal hypertension	M	68	1 92	170/110	8
086 P	Renal hypertension	M	53	1 82	215/130	7
087 P	Chronic coronary insufficiency	M	80	1 98	145/85	6
090 P	Myocardial infarction 3rd stage	M	73	1 63	140/95	7
	Mean		58 9	1 82		
	S D		12 4	0 15		

increase of the precordial counting rate (final level) was recorded and a blood sample withdrawn, to determine plasma volume and haematocrit

Cardiac output (CO) was calculated from the following formula

$$CO = \frac{\text{Blood volume (ml)} \times \text{final level (cpm)} \times \text{HF}}{\text{Area of the radiocardiogram (cpm} \times \text{min)}}$$

HF is the "Heart Fraction" (2) introduced to correct for the extracardiac activity contributing to the final level and averages 0 83 The area of RCG was completed by semilog extrapolation

Pulmonary circulation time ($\overline{PCT}$) was calculated in heart cycles using the mid time method (2—7) as the average of the minimum and maximum transit time

Pulmonary blood volume (PBV) was calculated as the product of $\overline{PCT}$ and stroke volume (SV)

The venous pressure was measured with an U shaped manometer filled with saline from an antecubital vein, taking as zero reference, a point 5 cm below the sternal notch

The enlargement of the left ventricle, judged on the standard postero anterior chest film, was classified as slight (+) evident (++) and marked (+++)

Material

Thirty three patients were studied (8 females and 25 males) aged from 32 to 80 years, admitted to the hospital at least a week before the study The group included 10 cases of hypertensive heart disease (HHD) with evident signs of left ventricular strain and positive indices of Lewis Jinich and Sokolow 14 cases of myocardial infarction (MI) in the second or third stage, 2 cases with recent coronary thrombosis (CT) and 7 cases with chronic coronary insufficiency (CCI) of varying degree The diagnosis was established according to anamnestic clinical and instrumental criteria None showed or had previously presented signs of heart failure

The patients were divided into 3 groups according to the size of the left ventricle as judged on standard postero anterior chest film

AP arterial pressure VP venous pressure BV = blood volume HR heart rate ci = c
blood volume

BV (ml)/m	HR (cycles/min)	c (l/min/m²)	SV (ml)	TCT (heart cycles)	PBV (ml)	PBV (ml/m²)	PBV (%)
2 470	76	3 5	75	6 0	456	280	11 3
2 934	6	3 1	74	7 0	518	283	9 8
2 275	72	3 0	63	5 5	347	234	10 5
2 938	80	3 6	84	6 0	506	269	9 2
2 596	65	3 4	65	5 0	425	262	10 1
2 690	64	3 3	99	5 5	545	286	10 6
2 642	74	3 3	94	6 5	611	286	10 8
2 729	0	2 6	77	6 0	462	224	8 2
2 487	71	2 9	69	6 5	414	244	9 8
2 71	91	3 4	76	6 5	494	273	10 1
2 642 3	72 9	3 21	79 7	6 05	477 8	264 6	10 0
215 9	5 5	0 3	11 0	0 6	74 3	23 1	0 9

standard chest film AP arterial pressure VP = venous pressure BV blood volume
la on me PBV pulmonary blood volume

BV ml m	HR (cycles min)	c (l/min/m²)	SV ml	TCT (heart cycles)	PBV ml)	PBV (ml/m²)	PBV (%)
2 330	80	3 0	67	6 0	402	225	9
2 537	72	2 5	65	6 5	422	225	8
2 117	71	2 9	74	7 0	518	288	11
2 3 8	73	2 5	60	7 5	450	2 7	11
2 934	68	2 2	51	10 0	510	321	12
2 722	64	2 4	68	7 5	510	280	10
2 6 4	56	2 6	75	—			—
2 780	94	3 0	6	10 0	560	326	11
693	74	2 6	67	9 0	603	317	11
2 44	60	3 0	84	5 5	462	277	11
2 831	74	2 9	67	6 0	402	236	8
23	56	2 5	82	6 5	533	290	10
2 484	9	2 6	71	6 0	426	263	10
2 671 4	0 0	2 68	68 2	4	483 2	275 4	10
187	12 7	0 28	9 4	1 1	65 4	35 5	1.

TABLE II Group II Patients with slight signs of left ventricular enlargement on standard chest film index, SV = stroke volume $\overline{PCT}$ = mean pulmonary circulation time PBV = pulmonary

Case	Diagnosis	Sex	Age	BSA (m²)	AP (mm Hg)	VP (cm H_2O)
014 P	Renal hypertension	F	46	1 63	240/160	6
027 P	Chronic coronary insufficiency	M	63	1 80	150/90	8
031 P	Chronic coronary insufficiency	F	64	1 48	160/90	7
038 P	Myocardial infarction 2nd stage	F	48	1 88	130/80	8
039 P	Coronary thrombosis	M	47	1 62	135/90	7
043 P	Renal hypertension	M	33	1 90	245/140	7
061 P	Renal hypertension	M	40	2 14	260/170	8
064 P	Myocardial infarction 2nd stage	M	66	2 06	100/60	5
066 P	Chronic coronary insufficiency	M	48	1 70	140/85	6
076 P	Coronary thrombosis	M	53	1 81	135/80	7
	Mean		50 8	1 80		
	S D		10 8	0 20		

TABLE III Group III Patients with evident or marked signs of left ventricular enlargement on heart rate CI = cardiac index SV = stroke volume $\overline{PCT}$ = mean pulmonary circu

Case	Diagnosis	Sex	Age	BSA (m²)	AP (mm Hg)	VP (cm H_2O)
008 P	Essential hypertension	M	54	1 79	230/125	—
024 P	Myocardial infarction 2nd stage	M	58	1 88	110/70	6
027 P	Angina pectoris	M	64	1 80	145/95	8
032 P	Angina pectoris[1]	M	64	1 75	140/85	7
041 P	2nd myocardial infarction 2nd stage	M	46	1 59	150/95	8
048 P	Myocardial infarction 3rd stage	M	59	1 82	135/85	8
049 P	Essential hypertension	F	49	1 61	200/110	8
056 P	2nd myocardial infarction 2nd stage	M	32	1 72	115/80	9
059 P	3rd myocardial infarction 3rd stage[1]	M	35	1 90	135/85	8
060 P	Myocardial infarction 3rd stage	M	57	1 67	140/80	—
072 P	Myocardial infarction 2nd stage	M	61	1 70	110/85	—
075 P	Myocardial infarction 3rd stage	M	65	1 84	150/90	8
088 P	Renal hypertension	F	57	1 62	195/125	9
	Mean		53 9	1 745		
	S D		10 6	0 10		

[1] Marked signs of left ventricular enlargement

the third group are consistently distributed below the regression line

PBV values in no case exceeded the extreme limits of the upper normal range However, when PBV is compared with the stroke volume (fig 3), the values in the first two groups appear to be distributed above the regression line for non cardiac subjects (7)

The changes in the relationship between PBV and SV seem to be of greater importance than those of PBV itself In fact PBV has been shown not to increase strikingly in various conditions associated with increased left atrial pressure as in mitral stenosis (1 3 4 8 10) or acute left ventricular failure (9)

For comparable degrees of obstruction to the outflow from the pulmonary veins it seems reasonable to assume that PBV will change according to the behaviour of the right ventricular output Even in presence of severe obstruction one may then anticipate that a low output will result in slight or no increase of PBV however the volume to flow ratio in the pulmonary vascular bed will be altered reflecting the fact that PBV values are out of proportion to the flow values

According to the relationship $\overline{PCT}$ PBV SV such a situation will be mirrored in an increase of the mean pulmonary circulation time $\overline{PCT}$

In fact when the distribution of CI PBV and $\overline{PCT}$ in the three groups are compared (fig 1) it is readily seen that the third group is characterized by an essentially normal PBV with a reduced CI resulting in an increased $\overline{PCT}$

On the other hand $\overline{PCT}$ shows only minimal variations around 6 heart cycles in cardiovascular normals and around 7 heart cycles in chronic obstructive emphysema (4, 5, 7) Values above 9 heart cycles may be observed in patients with mitral valve disease or left ventricular insufficiency, and in patients with paroxysmal tachycardia, while in the latter cases the SV is very low and PBV reduced (PBV/TBV $< 9\%$), in the former ones PBV is increased (PBV/TBV $> 13\%$) SV being normal or only slightly reduced (6)

It seems possible to conclude that when a lengthening of PCT occurs in association with PBV values in the upper normal range or above an elevation of left atrial pressure should be suspected

Such a condition is detectable in some of the cases of the third group, and it points out the presence of initial left ventricular failure

When one considers that these patients were not distinguishable either clinically or by X rays from the others of the same group, it seems reasonable to conclude that a wide clinical application of this technique may be of practical value for the assessment of compensation in presence of left ventricular enlargement and as a guide to treatment Even more so if one takes into account the easy performance and the little discomfort caused to the patients

Summary

Cardiac output and PBV were measured in 33 patients with coronary or hypertensive heart disease and various degrees

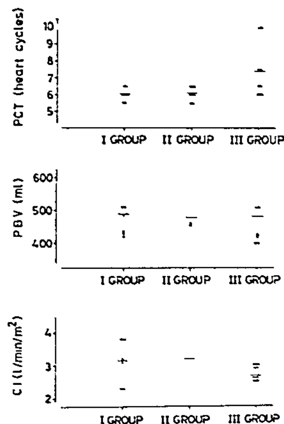


Fig 1 Values of CI PBV and $\overline{PCT}$ in the three groups of patients. The horizontal lines represent the group averages.

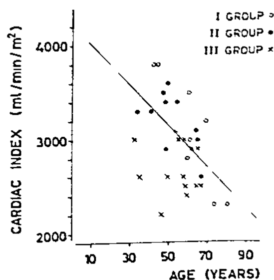


Fig 2 The values of CI in the three groups are plotted versus age The regression line was obtained in a group of normal subjects 7

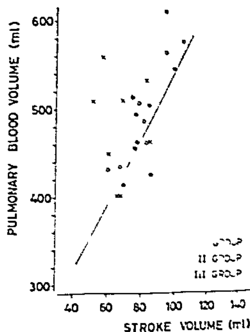


Fig 3 The values of SV are plotted versus PBV The regression line was obtained in a group of cardiovascular normals (7)

2nd stage and arterial hypotension and in patients with a low total blood volume and stroke volume, and showed the highest values in 3 patients with prolonged $\overline{PCT}$ (fig 1)

PBV was in the three groups respectively 9 95, 10 0 and 10 6 % of total volume This ratio showed the lowest values in 4 patients with myocardial infarction and hypotension 7 9 8 2, 8 9 and 8 3 %) and the highest in 3 subjects with lengthened PCT 12 5, 11 7 and 11 8 %)

Discussion

When the behaviour of the cardiac output in the three groups is considered in the light of the age distribution of the patients (fig 2 the values of CI in the first two groups are found to be scattered about the regression line for non cardiac patients 7) Conversely, the values in

the third group are consistently distributed below the regression line

PBV values in no case exceeded the extreme limits of the upper normal range However, when PBV is compared with the stroke volume (fig 3), the values in the first two groups appear to be distributed above the regression line for non cardiac subjects (7)

The changes in the relationship between PBV and SV seem to be of greater importance than those of PBV itself In fact PBV has been shown not to increase strikingly in various conditions associated with increased left atrial pressure as in mitral stenosis (1 3 4 8 10) or acute left ventricular failure (9)

For comparable degrees of obstruction to the outflow from the pulmonary veins it seems reasonable to assume that PBV will change according to the behaviour of the right ventricular output Even in presence of severe obstruction one may then anticipate that a low output will result in slight or no increase of PBV however, the volume to flow ratio in the pulmonary vascular bed will be altered reflecting the fact that PBV values are out of proportion to the flow values

According to the relationship $\overline{PCT}$ = PBV SV such a situation will be mirrored in an increase of the mean pulmonary circulation time $\overline{PCT}$

In fact when the distribution of CI PBV and $\overline{PCT}$ in the three groups are compared (fig 1) it is readily seen that the third group is characterized by an essentially normal PBV with a reduced CI resulting in an increased $\overline{PCT}$

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Such a condition is detectable in some of the cases of the third group, and it points out the presence of initial left ventricular failure

When one considers that these patients were not distinguishable either clinically or by X rays from the others of the same group it seems reasonable to conclude that a wide clinical application of this technique may be of practical value for the assessment of compensation in presence of left ventricular enlargement and as a guide to treatment Even more so if one takes into account the easy performance and the little discomfort caused to the patients

Summary

Cardiac output and PBV were measured in 33 patients with coronary or hypertensive heart disease and various degrees

of left ventricular enlargement, without clinical signs of left ventricular failure.

Only in 3 subjects with left ventricular enlargement evident (++) or marked (+++) a state of initial left ventricular failure was suggested by an alteration of the normal volume flow relationship, in pulmonary circulation, PBV being in the upper normal range.

The importance of this technique, as a clinical tool for the assessment of cardiac compensation, in presence of left ventricular enlargement, is stressed

Acknowledgement

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Plasma Lipid Fractions, Including Individual Phospholipids, at Various Stages of Pregnancy

By

Alvar Svanborg and Olle Vikrot

During pregnancy a pronounced hyperlipemia occurs In previous studies mainly cholesterol total phospholipids and total lipids have been measured and have been found to increase considerably In an earlier paper (72) preliminary observations on the plasma phospholipid composition showed a pronounced change during the last month of pregnancy Thus lysolecithin showed an absolute and relative decrease while the other phospholipids increased to a varying extent

The aim of the present investigation was to study the phospholipid pattern and the relationship between different lipid fractions at various stages of pregnancy The results were also compared with the corresponding findings in a study of healthy young women (31)

Material and methods

[illegible] pregnant women were studied once each during different stages of pregnancy usually at normal control visits Their ages ranged from 18 to 33 years with a mean of 25 years Eleven were nullparae eight I parae and two III parae

In addition two women were examined serially during pregnancy Subject A was a 23-year old woman who was pregnant for the first time and subject B a 36-year-old woman in her third pregnancy

All the women were healthy and the pregnancies were considered normal as judged by repeated clinical examinations with blood pressure measurements urine tests and hemoglobin determinations None showed excessive weight gain The duration of pregnancy was counted from the time of the last normal menstruation

Methods Blood was drawn between 8 and 10 a m and not under fasting conditions except for subject B

The collection of blood extraction of lipids and determination of phospholipids have been described previously (72) The extractions were performed in duplicate and determinations of phospholipids were done in duplicate on each extract From the same extracts suitable aliquots were also taken for determination of cholesterol and triglycerides

Cholesterol was determined by a modification of the ferric chloride method of Zlatkis et al (77) Usually 1 ml aliquots were evaporated and then saponified and extracted with petroleum ether (1 35) Color develop-

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of left ventricular enlargement, without clinical signs of left ventricular failure

Only in 3 subjects with left ventricular enlargement evident (++) or marked (+++) a state of initial left ventricular failure was suggested by an alteration of the normal volume-flow relationship, in pulmonary circulation, PBV being in the upper normal range

The importance of this technique, as a clinical tool for the assessment of cardiac compensation, in presence of left ventricular enlargement, is stressed

Acknowledgement

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TABLE III Plasma lipids in two pregnant women studied serially Symbols as in table I

Duration of pregnancy weeks	Triglycerides mM	Cholesterol mM	Total phospholipids mM	% of total P lipids				mM			
				PE	Lec	Sph	LL	PE	Lec	Sph	LL
Subject A											
9	0.72	4.04	2.54	2.6	70.5	22.8	4.2	0.07	1.79	0.58	0.11
14	0.80	4.82	3.03	3.1	71.5	21.9	3.5	0.10	2.17	0.67	0.11
18	0.50	4.73	3.19	4.9	70.5	21.8	2.8	0.16	2.25	0.70	0.09
22	1.07	5.43	3.32	3.3	72.9	21.8	2.0	0.11	2.42	0.72	0.07
27	1.07	6.55	3.31	3.8	71.0	23.6	1.6	0.13	2.35	0.78	0.05
31	1.58	6.35	3.70	4.2	71.4	22.8	1.6	0.16	2.64	0.84	0.06
35	1.87	6.75	3.75	4.7	70.8	23.1	1.5	0.18	2.65	0.86	0.06
Subject B											
9	0.37	5.10	2.86	2.7	69.0	23.7	4.7	0.08	1.98	0.68	0.13
14	0.70	5.65	3.39	3.3	70.6	23.7	2.5	0.11	2.39	0.80	0.08
21	1.28	7.35	3.93	3.9	69.9	24.4	1.8	0.15	2.75	0.96	0.07
25	1.38	7.10	4.11	4.7	70.7	23.0	1.6	0.19	2.91	0.95	0.07
30	1.92	7.31	4.27	5.1	70.7	21.9	2.4	0.22	3.02	0.94	0.10
34	2.76	8.54	4.44	5.1	72.3	21.2	1.3	0.23	3.21	0.94	0.06
37	2.57	7.86	4.29	5.7	74.0	18.1	2.3	0.24	3.17	0.77	0.10

TABLE IV Regression equations for various lipid fractions (Y) on time of pregnancy in weeks (X) in 21 women Symbols as in table I

Y		Equation	$s_{y \cdot x}$	s_b	r
Triglycerides	log (10 × mM)	$Y = 0.020X + 0.72$	0.115	0.0028	0.86
Cholesterol	mM	$Y = 0.077X + 4.31$	0.719	0.0177	0.71
Total phospholipids	mM	$Y = 0.057X + 2.49$	0.435	0.0107	0.77
PE	mM	$Y = 0.005X + 0.05$	0.034	0.0008	0.80
PE	% of P lipids	Y = 0.071X + 2.[illegible]	0.56	0.0137	0.77
Lec	mM	$Y = 0.044X + 1.72$	0.341	0.0084	0.77
Lec	% of P lipids	$Y = 0.095X + 69.7$	1.28	0.0315	0.57
Sph	mM	$Y = 0.009X + 0.60$	0.079	0.0019	0.73
Sph	% of P lipids	$Y = 23.2 - 0.086X$	1.07	0.0263	−0.60
LL	mM	$Y = 0.13 - 0.001X$	0.020	0.0005	−0.47
LL	% of P lipids	$Y = 4.7 - 0.081X$	0.76	0.0186	−0.71

Triglycerides were determined mainly according to Carlson (16) Single aliquots of 2 5 ml from each extract were evaporated, dissolved in 6 ml of chloroform and shaken with about 0.5 grams of silicic acid (Bio-Rad Laboratories Richmond California) activated at 120 C overnight before use After centrifugation duplicate 2 ml samples were

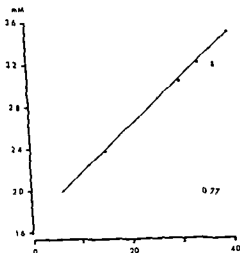
TABLE I Standard error for a single determination, calculated from 100 quadruplicate determinations PE = phosphatidylethanolamine Lec = lecithin Sph = sphingomyelin LL = lysolecithin

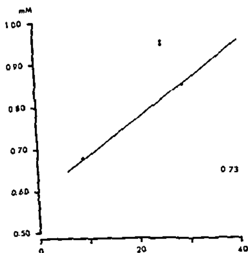
	Range	Mean	S E	S E (% of mean)
PE (% of total phospholipids)	0.7 – 6.9	4.0	0.36	9.0
Lec (% of total phospholipids)	64.7 – 76.1	71.0	0.70	1.0
Sph (% of total phospholipids)	16.8 – 26.0	21.7	0.60	2.8
LL (% of total phospholipids)	0.8 – 9.4	3.3	0.22	6.8
Total phospholipids (mM)	2.33 – 6.19	3.68	0.025	0.7
Cholesterol (mM)	3.75 – 9.33	6.03	0.084	1.4
Triglycerides (mM)	0.36 – 5.75	1.80	0.086	4.8

TABLE II Plasma lipids in 21 pregnant women Symbols as in table I

Duration of pregnancy weeks	Triglycerides mM	Cholesterol mM	Total phospholipids mM	% of total P lipids				mM			
				PE	Lec	Sph	LL	PE	Lec	Sph	LL
7	0.74	5.42	2.65	2.2	69.6	23.3	5.0	0.06	1.84	0.62	0.13
11	0.65	3.78	2.54	3.0	69.0	22.5	5.5	0.03	1.75	0.57	0.14
13	1.04	5.12	3.31	3.0	72.4	21.7	2.9	0.10	2.40	0.72	0.10
14	0.82	4.46	2.83	3.8	71.8	21.3	3.0	0.11	2.04	0.61	0.09
15	0.97	6.28	3.38	3.1	70.1	22.8	4.1	0.11	2.37	0.77	0.14
16	0.70	5.38	3.42	3.5	69.0	23.6	4.0	0.12	2.36	0.81	0.14
17	1.51	5.13	3.48	4.3	73.5	19.8	2.4	0.15	2.56	0.69	0.08
20	2.21	6.72	3.82	3.6	72.2	21.7	2.5	0.14	2.76	0.83	0.10
22	1.34	6.21	4.52	4.3	73.3	19.9	2.5	0.19	3.31	0.90	0.11
22	1.12	5.15	3.16	4.5	69.7	23.4	2.6	0.14	2.20	0.74	0.08
23	1.59	6.67	4.13	4.2	72.9	20.9	2.0	0.17	3.01	0.86	0.03
23	2.01	6.71	4.48	4.7	73.4	20.3	1.6	0.21	3.29	0.91	0.07
23	1.81	5.91	3.74	4.8	71.2	20.9	3.1	0.18	2.66	0.78	0.12
25	2.87	7.57	4.86	5.6	72.9	19.6	1.8	0.27	3.54	0.95	0.09
29	2.36	6.09	3.99	4.3	71.6	21.3	2.9	0.17	2.86	0.85	0.11
29	2.06	7.12	4.01	3.8	72.5	21.6	2.2	0.15	2.91	0.86	0.09
31	1.97	7.26	4.39	4.1	72.0	21.6	2.3	0.18	3.16	0.95	0.10
34	2.69	6.42	3.87	4.8	72.5	20.2	2.5	0.19	2.81	0.78	0.10
36	2.49	6.40	4.18	4.9	72.7	20.8	1.6	0.21	3.04	0.87	0.07
37	2.06	6.94	4.39	4.6	72.6	20.4	2.4	0.20	3.19	0.90	0.11
40	3.27	7.16	4.73	5.3	73.8	19.0	1.9	0.25	3.49	0.90	0.09

ment was performed according to Crawford (20) Duplicate aliquots were taken from each extract Blanks and standards of 50 and 100 micrograms were run on each occasion The values were referred to a standard prepared from cholesterol standard for clinical work (Nutritional Biochemicals Corp, Cleveland Ohio)





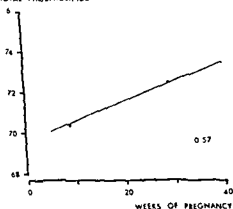


Fig 5

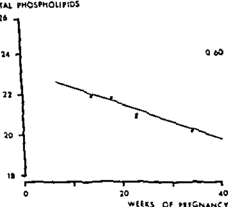


Fig 6

pipetted off and evaporated Then saponification and color development were carried out according to Carlson Tripalmitin B D H Poole England) was used as standard and was purified by silicic acid column chromatography and checked by thin layer chromatography before use On each occasion blanks and standards of 100 and 200 micrograms were taken through the procedure from the saponification step

The technical errors associated with the methods calculated from 100 quadruplicate determinations are presented in table I

Statistical methods Statistical calculations were made according to Snedecor (63) In the analysis, all calculations were made from the values in the main group of 21 women. The values from the serial subjects were excluded

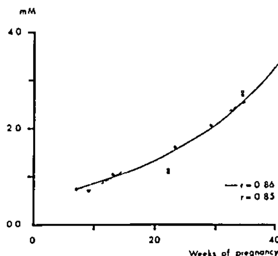
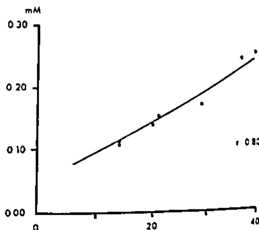


Fig 1

PHOSPHATIDYLETHANOLAMINE



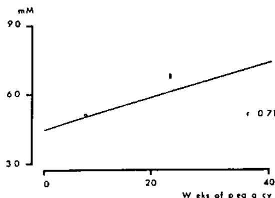


Fig 2

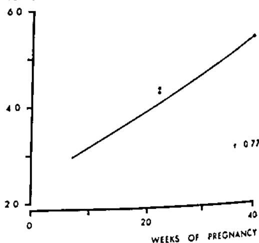


Fig 4

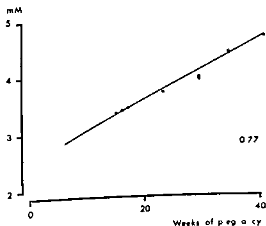


Fig 3

Figs 1—7 Changes in plasma lipids during pregnancy Regression equations and correlations were calculated from the main group of 21 pregnant women (●) In addition the values for serial subjects A (■) and B ( ) are shown In fig 1 two regression curves are shown the dashed line was calculated from unconverted values and the solid line after conversion to the logarithm In this figure r = 0 86 is the correlation coefficient between log (10 × triglycerides) and the duration of pregnancy

TABLE V Relationship between individual phospholipids and total amount of phospholipids in 21 pregnant women Regression coefficients and partial correlation coefficients Individual phospholipids were expressed as per cent of total phospholipids total phospholipids in mM b = regression coefficient for the regression of A on B r_{AB} = correlation coefficient between A and B $r_{AB\ T}$ = partial correlation coefficient between A and B keeping time of pregnancy constant. P is the level of significance of the correlation coefficients

A	B	b	r_{AB}	$r_{AB\ T}$
% phosphatidylethanolamine	Total phospholipids	0 98	0 78 p < 0 001	0 46 p < 0 05
% lecithin	Total phospholipids	1 09	0 74 p < 0 001	0 58 p < 0 01
% sphingomyelin	Total phospholipids	−1 42	−0 73 p < 0 001	−0 53 p < 0 05
% lysolecithin	Total phospholipids	−1 26	−0 81 p < 0 001	−0 58 p < 0 01

TABLE VI Relationship between different lipid fractions in 21 pregnant women Regression coefficients correlation coefficients and partial correlation coefficients Values in mM Calculations of triglycerides were made on log (10 × molar concentration) $r_{AB\ TC}$ = partial correlation coefficient between A and B with time of pregnancy and the concentration of the third variable kept constant Other symbols as in table V

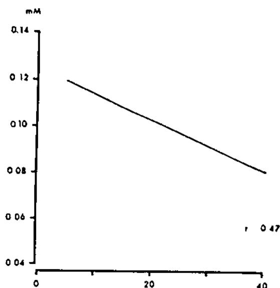
A	B	b	r_{AB}	$r_{AB\ T}$	$r_{AB\ TC}$
Log (10 × triglycerides)	Cholesterol	0 176	0 81 P < 0 001	0 56 P < 0 05	0 27 P < 0 1
Log (10 × triglycerides)	Phospholipids	0 271	0 84 P < 0 001	0 54 P < 0 05	0 23 P < 0 1
Cholesterol	Phospholipids	1 31	0 88 P < 0 001	0 75 P < 0 001	0 64 P 0 01

The correlation coefficients between percentages of different phospholipid fractions and total phospholipids were significant as were the partial correlation coefficients when time was kept constant (table V)

When total phospholipids cholesterol and log (10 triglycerides) were compared pairwise with each other the correlation coefficients were significant. Partial correlation coefficients with time kept constant were also significant With time and one of the three fractions kept constant the partial correlation coefficient between cholesterol and phospholipids was still significant but not those between log (10 × triglycerides) and either cholesterol or phospholipids (table VI)

Discussion

Plasma lipids and duration of pregnancy The values for cholesterol and total phospholipids obtained in this study



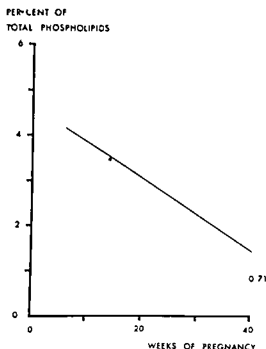


Fig 7 Text on page 618

To study the variation of different lipid fractions with duration of pregnancy, a linear regression was assumed, except in the case of triglycerides In non pregnant women it has been shown that the distribution of triglyceride values is not symmetrical, and the assumption was made that log (10 × triglycerides) is approximately normally distributed as no significant asymmetry was found (31) In the present study a similar distribution was assumed and thus a regression of the linear type log Y = a + b X was assumed, where Y = (10 × triglycerides) and X = time of pregnancy in weeks To study the increase of cholesterol with pregnancy, several non linear regression curves were tested but none gave a significantly better fit than the straight line in this material

The relationship between different lipid fractions was studied with the aid of correlation coefficients To eliminate the influence of the time of pregnancy, partial correlation coefficients were also studied

To test the null hypothesis of no correlation the t test was used All tests of significance were made at the 5 per cent level

Results

The plasma lipid values for the main group are presented in table II and for the serial subjects in table III

The regression equations for lipid fractions on time of pregnancy are presented in table IV All regression coefficients differed significantly from zero The graphs of the different regression equations are shown in figs 1—7 The values from the serial subjects are also shown in the figures and it is seen that the changes conformed rather well to the changes in the main group

All lipid concentrations, except that of lysolecithin, increased with time and were, at the end of pregnancy, well above non pregnant levels (31) Lysolecithin was the only lipid to decrease during pregnancy When phospholipids were calculated as the percentage of total phospholipids, lysolecithin and also sphingomyelin decreased, while the percentages of lecithin and phosphatidyl ethanolamine increased

TABLE V Relationship between individual phospholipids and *total amount* of phospholipids in 21 *pregnant women Regression* coefficients and partial correlation coefficients Individual phospholipids were expressed as per cent of total phospholipids total phospholipids in mM b = regression coefficient for the regression of A on *B* r_{AB} = correlation coefficient between A and B $r_{AB\,T}$ = partial correlation coefficient between A and B keeping time of pregnancy constant P is the level of significance of the correlation coefficients

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TABLE VI Relationship between different lipid fractions in 21 pregnant women Regression coefficients correlation coefficients and partial correlation coefficients. Values in mM Calculations of triglycerides were made on log (10 × molar concentration) $r_{AB\,TC}$ partial correlation coefficient between A and B with time of pregnancy and the concentration of the third variable kept constant Other symbols as in table V

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Cholesterol	Phospholipids	1 31	0 88 P < 0 001	0 75 P < 0 001	0 64 P 0 01

The correlation coefficients between percentages of different phospholipid fractions and total phospholipids were significant as were the partial correlation coefficients *when time was kept* constant (table V)

When total phospholipids cholesterol and log 10 × triglycerides, were compared pairwise with each other the correlation coefficients were significant Partial correlation coefficients with time kept constant were also significant With time and one of the three fractions kept constant the partial correlation coefficient between cholesterol and phospholipids was still significant but not those between *log (10 ×* triglycerides) and either cholesterol or phospholipids (table VI)

Discussion

Plasma lipids and duration of pregnancy The values for cholesterol and total phospholipids obtained in this study

agree rather well with those found in other investigations. However, the mode of rise has been somewhat different in various series. Oliver and Boyd (47), Watson (73), De Alvarez et al. (21) and Pantelakis et al. (48) found that the cholesterol level increased progressively during pregnancy but decreased before delivery, or reached a constant level during the last trimester. Oliver and Boyd (47) observed a maximum at about the 33rd week and Watson (73) at the 29th week of pregnancy. For the phospholipids, Oliver and Boyd (47) and Pantelakis et al. (48) found a similar decrease before term but De Alvarez et al. (21) observed a continuous increase until delivery.

Other authors, e.g. Moses et al. (43) and Vernet and Smith (71) for cholesterol and Peters et al. (49) for cholesterol and phospholipids, have found a continuous increase up to term. Von Studnitz (66), in the largest published material, found a quite linear increase up to term for both fractions. In another paper (68) the blood lipids were analyzed in six women at the onset of labor. The levels were found to be close to those calculated from the regression equations in the present study. The discordancy in the results of the different investigators is difficult to explain.

Triglycerides showed the greatest percentage increase during pregnancy. For the statistical calculations of the triglyceride values, the logarithmic conversion was used, since it has been found in non-pregnant individuals that the absolute values of triglycerides show a skew distribution which becomes more symmetrical after conversion to the logarithm (15, 24, 31). It is not certain that such a log normal distribution is found also during every stage of the pregnancy, and the present material does not allow of conclusions on this question. The assumption of a log normal distribution of triglycerides during the whole of pregnancy seems, however, more reasonable than a normal distribution. In the present material the regression of log (10 × triglycerides) with respect to the time of pregnancy appeared to be linear, which would indicate that triglycerides increase exponentially. This must, however, be a tentative conclusion, as the exponential curve differs only slightly from the straight line fitted to the unconverted values according to the principle of least squares (fig. 1).

The plasma triglycerides in pregnancy have not generally been analyzed with direct methods. However, in some serial studies values for "neutral fat" have been determined and this fraction is mainly triglycerides. Thus Schwarz et al. (61) and Peters et al. (49) found a continuous increase which was more marked than that of the other lipid fractions. With methods similar to that used here, Kontinen et al. (34) and Cramer et al. (19) have found values at the end of pregnancy well in agreement with those of the present study.

The change in phospholipid composition during pregnancy was quite similar to that reported previously (72), except that the present larger material clearly showed that the sphingomyelin percentage decreased, which was not evident in the previous study. On an absolute basis, however, sphingomyelin increased as did

lecithin and phosphatidylethanolamine It is remarkable that while all other lipid fractions increased, the lysolecithin showed a marked decrease As the average increase in plasma volume during pregnancy has been reported to be about 50 per cent (30), the decrease in lysolecithin might be due partly to a hemodilution However, since the concentration of lysolecithin went down about 50 per cent, hemodilution could explain only a part of the decrease

Interrelations between lipids Cholesterol increased more than phospholipids The regression of cholesterol (Y) on phospholipids (X) was $Y = 1.31 X + 1.12$ The intercept with the y axis did not differ significantly from zero and thus the regression line can be considered as essentially passing through the origin It can be seen that therefore the cholesterol/phospholipid ratio did not change during pregnancy Oliver and Boyd (47) found a pronounced increase in this ratio during pregnancy but this has not been a general experience Thus Schwarz et al (61) and Peters et al (49) found no consistent change and calculation of this ratio from other publications (21 48 66) does not show a distinct rise The results in the present study thus agree with the majority of investigations No explanation can be given for the discrepant findings of Oliver and Boyd The regression of cholesterol on phospholipids was not significantly different from that obtained in a study of young non pregnant women (31) where the equation was $Y = 1.74 X - 0.17$

The correlations between log (10 × triglycerides) on the one hand and phospholipids or cholesterol on the other were significant in the pregnant women but not significant in the non pregnant women In order to investigate whether the non pregnant group differed from the pregnant group, the following procedure was used (74) The regression equations for log (10 × triglycerides) on phospholipids, and on cholesterol were calculated in the pregnant group For every individual in a group of 23 non pregnant women (31) a value for log (10 × triglycerides) was calculated by inserting the value for total phospholipids or cholesterol in these regression equations The difference between the observed and the calculated values for log (10 × triglycerides) was analyzed by the t test These differences were negative and significantly different from zero (P < 0 001) in both cases

Thus in the non pregnant group the relationship between triglycerides and phospholipids and between triglycerides and cholesterol was such that for any given phospholipid or cholesterol value the triglyceride level was lower for the non pregnant than for the pregnant women No similar calculations on the lipemia of pregnancy have been published previously However several authors (11 49 61) have pointed out that neutral fat is the fraction which shows the greatest increase

The mechanism of hyperlipemia during pregnancy The more pronounced rise in plasma triglycerides may indicate that the mechanism behind the triglyceride increase partly differs from that regulating the level of cholesterol and phospholipids The women in this investigation were generally not fasting while the women in the non pregnant control

agree rather well with those found in other investigations However, the mode of rise has been somewhat different in various series Oliver and Boyd (47), Watson (73), De Alvarez et al (21) and Pantelakis et al (48) found that the cholesterol level increased progressively during pregnancy but decreased before delivery, or reached a constant level during the last trimester Oliver and Boyd (47) observed a maximum at about the 33rd week and Watson (73) at the 29th week of pregnancy For the phospholipids, Oliver and Boyd (47) and Pantelakis et al (48) found a similar decrease before term but De Alvarez et al (21) observed a continuous increase until delivery

Other authors, e g Moses et al (43) and Vernet and Smith (71) for cholesterol and Peters et al (49) for cholesterol and phospholipids, have found a continuous increase up to term Von Studnitz (66), *in the largest published material, found* a quite linear increase up to term for *both fractions In another paper (68) the* blood lipids were analyzed in six women at the onset of labor The levels were found to be close to those calculated from the regression equations in the present study The discordancy in the results of the different investigators is difficult to explain

Triglycerides showed the greatest *percentage* increase during pregnancy For the statistical calculations of the triglyceride values, the logarithmic conversion was used, since it has been found in non-pregnant individuals that the absolute values of triglycerides show a skew distribution which becomes more symmetrical after conversion to the logarithm (15, 24, 31) It is not certain that such a log normal distribution is found also during every stage of the pregnancy, and the present material does not allow of conclusions on this question The assumption of a log normal distribution of triglycerides during the whole of pregnancy seems, however, more reasonable than a normal distribution In the present material the regression of log (10 $\times$ triglycerides) with respect to the time of pregnancy appeared to be linear, which would indicate that triglycerides increase exponentially This must, however, be a tentative conclusion, as the exponential *curve differs only slightly* from the straight line fitted to the unconverted values according to the principle of least squares (fig 1)

The plasma triglycerides in pregnancy have not generally been analyzed with direct methods However, in some serial *studies values for "neutral fat"* have been determined and this fraction is mainly *triglycerides Thus Schwarz* et al (61) and Peters et al (49) found a continuous increase which was more marked than that of the other lipid fractions With methods similar to that used here, Konttinen et al (34) and Cramer et al (19) have found values at the end of pregnancy well in agreement with those of the present study

The change in phospholipid composition during pregnancy was quite similar to that reported previously (72), except that the present larger material clearly showed that the sphingomyelin percentage decreased, which was not evident in the previous study On an absolute basis, however, sphingomyelin increased as did

The change in the phospholipid composition cannot be a general consequence of hyperlipemia In nephrosis, for example there is usually an increase of lysolecithin and sphingomyelin with a relative decrease in lecithin (45), and in one family with a hereditary hyperlipemia all phospholipids increased to the same extent except sphingomyelin which remained unchanged (17)

In pregnancy profound endocrine changes take place and hormonal factors have often been considered as the cause of the hyperlipemia The effect of hormones on individual phospholipids is largely unknown Jensen (32) studied the effect of stilbestrol on hypercholesterolemic men and found that alkali labile phospholipids (mainly phosphoglycerides) increased while alkali-stable phospholipids (mainly sphingomyelin) did not show a significant rise Lysolecithin was not measured The relative decrease of sphingomyelin is in accordance with the findings in pregnancy but an absolute increase of sphingomyelin was found by Jensen only in some of the cases Lyman et al (40) found no effect on the phospholipid pattern in castrated rats which were treated with small physiological doses of estradiol but the effect of large doses comparable to the amounts produced during pregnancy was not studied In a recent paper (27) the effect of small doses of conjugated equine estrogens on the serum phospholipids in post menopausal women was reported The increase of total phospholipids was due to an increase of lecithin while sphingomyelin and lysolecithin showed a tendency to decrease and phosphatidylethanolamine was unchanged Thus, the change in the phospholipids was in the same direction as that seen in pregnancy It is possible that estrogens are involved in the production of pregnancy hyperlipemia While in most studies on hyperlipemic persons estrogens decreased the cholesterol level, Berezin and von Studnitz (8) found a rise in several women with normal levels prior to the treatment

The placenta may contribute to the changes in plasma lipids by selective production or uptake There is no evidence for production of plasma phospholipids in the placenta In the rabbit (which however shows lipopenia during pregnancy) Popják and Beeckmans (53) showed an active uptake of plasma phospholipids in the placenta To explain the change in the phospholipid pattern a larger uptake of lysolecithin than of the other phospholipids has to be assumed Eberhagen (22) found no lysolecithin in human placentas but a rather large amount of lysophosphatidylethanolamine The metabolism of lysolecithin in the placenta is not known but Winkler (75) found a lower activity of lysophosphatidase in placenta than in the liver of the rat Acylation of lysolecithin in the placenta has not been reported Available information thus does not support a selective uptake of lysolecithin by the placenta However further studies in this field are needed

The liver plays a central role in lipid metabolism, and the main part of the plasma lipids are produced there A low serum concentration of lysolecithin, similar to that found in pregnancy, has been observed in liver diseases especially in biliary cirrhosis (25 52) Other

group were in a post-absorptive state The "extra" rise in triglycerides could therefore be due to the nutritional state However, the findings of Schilling et al (60) show that a light breakfast including up to 17 grams of fat causes little change in the post-alimentary level of triglycerides in non pregnant individuals The post-heparin lipoprotein-lipase activity has been reported to be lowered in pregnancy (42, 58), and therefore the possibility exists that the alimentary lipemia is more pronounced in pregnant women However, according to Effkemann (23), a fat meal in pregnancy gives less hyperlipemia than in non-pregnant women

Cholesterol should not be influenced by an ordinary breakfast (60) Total phospholipids rise only little, even after fat loading (5) With regard to the effect of food on the phospholipid composition, unpublished observations by the authors showed only small and inconsistent changes during pronounced alimentary lipemia Marinetti et al (41) found similar compositions in two fasting and two non fasting subjects, and Robinson and Phillips (56) reported values for 8 healthy non fasting persons which were in agreement with those reported in the literature for fasting persons It thus seems reasonable to suppose that the cholesterol and phospholipid values in the present study did not differ much from what would be found in fasting pregnant women

The fact that women alter their total caloric intake and perhaps also the composition of the food when pregnant may contribute to the hyperlipemia, since nutritional factors are known to influence even the post absorptive level of blood lipids (2) In studies on pregnant rats, drastic changes in the diet have produced changes mainly in the triglyceride level, less in cholesterol and phospholipids (33, 62) In human pregnancy Moses et al (43) found that daily addition of 2 grams of cholesterol to the diet did not influence serum cholesterol or phospholipids, and Hansen et al (28) found no correlation between the levels of serum cholesterol or total fatty acids and the intake of calories, fat or protein in the third trimester The effect of a maintained increase in food intake on the phospholipid composition is not known It seems reasonable to assume that nutritional factors are not the main cause of pregnancy hyperlipemia but may be a contributory factor

Some further mechanisms could be responsible for the more pronounced rise in triglycerides than in cholesterol and phospholipids Bleicher et al have discussed a lipolytic factor in the placenta (9) and "accelerated starvation" (10) as etiological factors Laron and Kowadlo Silbergeld (39) found a fat mobilising effect of estrogens Some authors (10, 14, 36) though not all (34) have found increased levels of free fatty acids in pregnant women An increased uptake of such acids in the liver leads to increased formation of triglycerides and hyperglyceridemia (29, 65)

The hyperlipemia of pregnancy is especially characterized by the change in the composition within the phospholipid fraction In the following discussion of the mechanisms leading to this hyperlipemia, processes which might influence the phospholipids in this way will be considered especially

general phenomenon in pregnancy, and can be responsible for at least some part of the hyperlipemia In more pronounced cases it can also be responsible for pruritus and in the most severe instances for the recurrent jaundice of pregnancy (67)

To elucidate the mechanism of pregnancy hyperlipemia further studies are needed Hyperlipemia per se is common to many conditions The changes in the phospholipid pattern should be of help in future investigations as it serves, at least to some extent to distinguish the plasma lipid alterations in pregnancy from most other hyperlipemias

The phospholipids are important structural components in all organs The role of the phospholipids in plasma is not understood The remarkable changes in the relative and absolute concentration of the highly surface active lysolecithin such as occur in pregnancy, can be expected to influence the physico chemical state of all of the components of the blood

Summary

The levels of individual plasma phospholipids were analyzed in 21 healthy pregnant women at various stages of pregnancy In another 2 women serial analyses were made during the whole of pregnancy

The changes in the phospholipids were compared with the changes in cholesterol and triglycerides

All plasma lipid fractions increased with the duration of pregnancy except for the lysolecithin level which decreased Sphingomyelin increased relatively less than the total phospholipid level, thus showing a relative decrease within the phospholipid fraction

Significant correlations were obtained between the lipid fractions and the duration of pregnancy

The mechanism of the hyperlipemia of pregnancy is discussed

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findings in pregnancy lipemia are also similar to those in liver disease (3, 4, 50, 51) increase of total phospholipids, triglycerides, cholesterol and lecithin with a lowered percentage of sphingomyelin While it is usually considered that the cholesterol rise in hepatic disorders is mainly in the unesterified fraction, which is not the case in pregnancy (21), Zieve (76) showed that it was only in patients with jaundice that the percentage of cholesterol esters decreased The post-heparin lipoprotein-lipase activity is decreased during pregnancy (42, 58), which is also the case in hepatitis and obstructive jaundice (57), and this decreased activity might contribute to the increase of triglycerides

It has been suggested previously by some authors that the cause of the hyperlipemia of pregnancy is a change in liver function with a retention of bile (7, 54, 59), a hypothesis well worth further study Of interest in this connection is the observation that patients with pruritus of pregnancy and also other signs of biliary stasis have been reported to have higher concentrations of cholesterol and phospholipids in blood than other pregnant women (6)

Many observations show that there are alterations in liver function in pregnancy Thus, Combes et al (18) showed a tendency to retention of sulfobromophthalein, possibly caused by the high production of estrogens (44) The level of alkaline phosphatase increases during pregnancy (70) While this may be due partly to the production of enzyme in the placenta (12), increased phosphatase activity was also produced by high doses of estrogens (44) Serum bilirubin is often slightly increased and it has been reported that injected bilirubin is eliminated more slowly during pregnancy (64) The amount and composition of bile during pregnancy has not been thoroughly studied, but some investigations indicate that there may be a reduced concentration of cholesterol and phospholipids in bile during pregnancy with a rise after delivery (38, 54) It seems possible that these changes in liver function are caused by the increased steroid production during pregnancy

These findings indicate changes in the bile secretion during pregnancy In extra- or intrahepatic biliary stasis hyperlipemia is a common finding (3, 4, 51, 52), the mechanism of which has not been established The lowering of serum lysolecithin in liver disease was considered by Phillips (52) to be due to decreased synthesis It has, however, not been shown that serum lysolecithin is formed in the liver It may be produced in the blood stream by an enzyme which transfers fatty acids from lecithin to cholesterol and thus forms lysolecithin and cholesterol esters The enzyme, which has been studied by Glomset (26) among others, seems to be formed in the liver (13) The activity of the transferase enzyme was not lowered in plasma obtained from pregnant women (69) Further processes that could be responsible for low lysolecithin levels are increased breakdown or increased acylation to lecithin which is known to occur in the liver (37), in erythrocytes (46, 55) and in other organs

It seems reasonable to conclude that an alteration in hepatic function is a

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Plasma Lipids during the First Week after Delivery

By

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During pregnancy plasma lipids increase considerably (5 9 11 12, 14 17 19) How soon after delivery the hyperlipemia disappears is not known with certainty It has been reported that blood lipids can be found to be above normal levels several months post partum (5 11 12 14 19) However, pregnant lipid values were not generally known and due to the wide individual scatter of plasma lipids statements on the duration of pregnancy hyperlipemia are difficult to evaluate

Several observations indicate that the main plasma lipid fractions decrease considerably during the first post partum week (2 4 9 14 19) No studies have been published on the rate at which the individual phospholipids return to normal The change in the phospholipid pattern seems to be the most characteristic finding in pregnancy hyperlipemia (17 18) It was therefore considered to be of importance to follow the individual phospholipids during the first days after the delivery The present report includes repeated analyses of the plasma lipids during the first week

Submitted for publication May 17 1965

Material and methods

Six women were investigated They were healthy as judged by regular clinical examination during the pregnancy Their ages ranged from 19 to 42 years The course of the pregnancy was normal in all cases Five were delivered 39—41 weeks after the last menstruation One woman was not certain of the time of her last menstruation but all of the children were full term (weight 3 440—4 240 grams) The deliveries were normal Subject 5 lost an estimated 550 ml of blood at the delivery of the placenta and her hematocrit dropped from 38 per cent before delivery to 31—34 per cent on the following days She did not however receive any blood transfusion

The first blood specimen was taken at the beginning of labor and on further determinations were made at least 4 days of the first week after delivery while the patients were still in hospital Blood was drawn between 8 and 10 a m The subjects were not fasting

Collection and extraction of blood and methods for analysis of total and individual phospholipids cholesterol and triglycerides

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Collection and extraction of blood and methods for analysis of total and individual phospholipids cholesterol and triglycerides

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TABLE I Plasma lipids in 6 normal women at delivery and during the first post partum week PE= phosphatidylethanolamine Lec=lecithin Sph=sphingomyelin LL=lysolecithin

Subject	Days post partum	Triglycerides mM	Cholesterol mM	Total phospholipids mM	% of total P lipids				mM			
					PE	Lec	Sph	LL	PE	Lec	Sph	LL
1	0	2 02	6 05	3 76	5 6	71 1	22 3	1 1	0 21	2 67	0 84	0 04
	1	1 93	5 57	3 43	5 0	70 5	22 5	1 9	0 17	2 42	0 77	0 07
	2	1 67	5 45	3 48	4 1	70 9	22 8	2 2	0 14	2 47	0 79	0 08
	3	1 36	5 30	3 45	3 5	68 9	23 9	3 6	0 12	2 38	0 83	0 13
	4	0 91	5 27	3 24	2 9	70 0	23 5	3 6	0 09	2 27	0 76	0 12
2	0	3 72	6 39	4 13	5 9	73 8	19 3	1 0	0 24	3 05	0 80	0 04
	1	2 98	5 45	3 44	4 7	72 5	21 0	1 8	0 16	2 49	0 72	0 06
	2	2 90	5 09	3 27	5 0	71 3	21 6	2 1	0 16	2 33	0 71	0 07
	3	3 01	5 50	3 80	4 3	71 2	21 2	3 3	0 16	2 70	0 81	0 13
	4	2 38	5 03	3 42	3 9	72 1	20 7	3 2	0 14	2 47	0 71	0 11
3	0	3 02	6 53	4 31	5 0	71 8	22 3	0 9	0 22	3 10	0 96	0 04
	1	2 06	5 88	3 79	4 2	71 6	23 1	1 1	0 16	2 71	0 88	0 04
	2	2 30	6 34	4 24	4 2	70 9	23 1	1 7	0 18	3 00	0 98	0 07
	3	1 46	5 63	3 60	3 3	69 5	24 1	3 2	0 12	2 50	0 87	0 11
	4	1 58	6 10	3 84	3 7	69 5	23 6	3 2	0 14	2 67	0 91	0 12
	5	1 73	6 63	4 04	3 5	70 2	24 0	2 3	0 14	2 84	0 97	0 09
	7	1 17	6 23	3 61	3 6	69 8	24 4	2 3	0 13	2 52	0 88	0 08
4	0	2 95	5 67	3 92	5 3	75 1	18 6	1 0	0 21	2 94	0 73	0 04
	1	2 70	4 79	3 61	5 2	74 2	18 7	1 9	0 19	2 68	0 67	0 07
	2	2 34	5 07	3 65	4 2	74 5	18 8	2 6	0 15	2 72	0 69	0 09
	3	2 00	5 45	3 66	3 6	74 4	18 9	3 1	0 13	2 72	0 69	0 11
	4	1 09	4 92	2 99	3 0	74 4	20 3	2 3	0 09	2 22	0 61	0 07
	6	1 69	5 30	3 17	2 7	72 2	22 0	3 1	0 09	2 29	0 70	0 10
5	0	5 48	7 97	5 04	6 5	75 5	16 8	1 2	0 33	3 81	0 85	0 06
	1	3 91	6 30	4 01	5 6	75 1	17 1	2 1	0 23	3 01	0 69	0 08
	2	4 40	5 88	4 19	5 6	75 7	16 2	2 5	0 23	3 17	0 68	0 10
	3	3 61	6 11	3 99	4 6	74 5	17 7	3 3	0 18	2 97	0 71	0 13
	5	3 41	6 09	3 69	4 8	74 7	18 0	2 5	0 18	2 76	0 66	0 09
6	0	3 71	9 24	5 41	5 2	74 1	19 2	1 5	0 28	4 01	1 04	0 08
	1	2 43	8 28	4 45	5 0	71 9	21 6	1 6	0 22	3 20	0 96	0 07
	2	2 83	7 89	4 35	5 0	72 2	21 0	1 9	0 22	3 14	0 91	0 08
	3	2 52	7 19	3 99	4 9	71 5	20 9	2 7	0 20	2 85	0 83	0 11
	5	1 76	7 66	4 09	3 4	72 9	20 8	3 0	0 14	2 98	0 85	0 12

have been described earlier (17 18) Statistical calculations were made according to Snedecor (15)

Results

The plasma lipid values are presented in table I Table II gives the mean difference between the initial values (Day 0) and the values on the first post partum day (Day 1) as well as the difference between Day 0 and the fourth or the fifth post partum day

On the first day after delivery there was an obvious change in the direction of non pregnant values (6) Thereafter the changes were slower and with some fluctuations but in almost all instances further changes towards normal values were observed

The changes in the phospholipid composition were rather pronounced with e g an increase of lysolecithin from 1 to 3 per cent of the total phospholipids in 4 days Lysolecithin was the only individual lipid which showed an absolute rise during this period The most pronounced decrease on a molar basis was in the triglyceride fraction which was approximately halved during the first four days

At the time of the last blood samples the values had not returned to non pregnant levels

Discussion

The plasma lipid values of the patients in labor were not significantly different from those calculated for the end of pregnancy (table III) and it seems unlikely that any important change in lipid levels occurs just before delivery Luukkainen and Csapo (10) and also Jaisle (7, 8) have suggested that phospholipids might in some way be involved in the initiation of labor, for infusions of phospholipids sensitize the pregnant rabbit uterus to oxytocin While it is possible that e g changes in uterine phospholipids could be responsible, it eems that in any case plasma phospholipids are not involved in the initiation of labor in humans

Immediately post partum there is a decrease in plasma volume (1, 3) which may influence blood lipid levels There seem to be wide individual variations in the rate of change In the present study plasma volume was not measured, but calculation of pos ible changes due to hemoconcentration indicates that the maximal increase due to this factor would be about 50 per cent above the initial level

The decreases in cholesterol, triglycerides and total phospholipids in this study were of the same order of magnitude as described by others (2, 9, 14 19) Konttinen et al (9) found triglycerides to decrease about 50 per cent during the first five days after delivery while total phospholipids decreased about 15 per cent and total cholesterol about 5 per cent The data of Oliver and Boyd (11) seem to be somewhat divergent and show an initial decrease of cholesterol and phospholipids and then on the 7th day an increase up to or above values at delivery The reasons for this discrepancy are not known

Dannenburg et al (4) in their serial study of 10 women in the early puerperium separated out 3 lipid classes on

TABLE II Changes in plasma lipids between indicated days after delivery (Values given are means of differences for subject 3 a mean of values on day 4 and 5 was used)

	Triglycerides mM	Cholesterol mM	Total phospholipids mM
Day 0 — Day 1	−0.82	−0.93	−0.64
S E M	0.235	0.167	0.126
Day 0 — Day 4 or 5	−1.62	−1.08	−0.87
S E M	0.161	0.259	0.167

silicic acid columns and then measured ester bonds with tripalmitin as standard Their absolute values are difficult to compare with those in the present study, but they also found the greatest change in triglycerides, less in phospholipids and least in esterified cholesterol These authors reported an increase especially of triglycerides on the first post-partum day, which however was not significant Such an increase was not found in any of the six women studied here

It is obvious that as early as the day after delivery lysolecithin began to increase in concentration while the other phospholipids began to decrease The increase in lysolecithin may be due partly to hemoconcentration, but the absolute increases of lysolecithin during the period of study were of the order of magnitude of 100 per cent of the initial value, so that there must also have been a real increase

If the lipid metabolism within the placenta were directly responsible for the hyperlipemia of pregnancy a rapid normalization after delivery would be expected The present data, which show

TABLE III Mean values for plasma lipids in 6 women on the day of delivery compared with the value calculated for 40 weeks of pregnancy according to regression equations in the paper of Svanborg & Vikrot (17) Symbols as in table I S E M = standard error of the mean Values for triglycerides were calculated after conversion to logarithms and then reconversion

	Triglycerides mM	Cholesterol mM	Total phospholipids mM	% of total P lipids				mM			
				PE	Lec	Sph	LL	PE	Lec	Sph	LL
Mean value	3.33	6.98	4.43	5.6	73.6	19.8	1.1	0.25	3.26	0.87	0.05
S E M	—	0.55	0.27	0.22	0.72	0.89	0.09	0.02	0.21	0.05	0.01
Calculated value	3.31	7.39	4.77	5.3	73.5	19.8	1.5	0.25	3.48	0.96	0.09

for six women with standard errors of the means (S E. M) Symbols as in table I (For calculation

% of total P lipids				mM			
PE	Lec	Sph	LL	PE	Lec	Sph	LL
−0 6	−0 9	+0 9	+0 6	−0 06	−0 51	−0 09	+0 02
0 16	0 29	0 37	0 15	0 012	0 103	0 015	0 006
−2 0	−1 3	+1 5	+1 8	−0 12	−0 69	−0 11	+0 0[illegible]
0 18	0 19	0 09	0 21	0 011	0 124	0 026	0 008

a progressive change towards non pregnant levels indicate that such a direct effect of the placenta can hardly be the only mechanism

The placenta and the fetus are the ultimate cause of the hyperlipemia The relatively slow changes in plasma lipids after delivery are not evidence against the assumption that the hyperlipemia is due to a cause such as the hormones produced by the placenta At delivery there is an abrupt drop in this hormone formation but it is not known how long such a presumed hormonal effect on blood lipids would last

If the alterations of the liver metabolism during pregnancy were one mechanism for the hyperlipemia (17), it would be expected from clinical experience and from e g the findings of Petersen (13) that only some time after delivery would there be normalization of the liver function The gradual changes in cholesterol and phospholipids during the early puerperium are not in conflict with such a theory Here it should be noted that abnormalities in liver function tests after recurrent jaundice of pregnancy do not disappear until some weeks after delivery (16)

Summary

Plasma lipids including individual phospholipids, were determined in 6 pregnant women on the day of delivery and then repeatedly during the first puerperal week

The lipid levels on the day of delivery agreed well with those calculated from regression equations in normal pregnancy

As early as the first post partum day an obvious change towards non pregnant levels was noted Thereafter the changes were slower At the time of the last blood samples (4—7 days after delivery) the levels and also the phospholipid composition had not yet returned to non pregnant values

Acknowledgement

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Individual Plasma Phospholipids in Healthy Young Women

By

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The relationship between the concentrations of the various plasma lipid fractions has been a field of active study Most investigators have been concerned with the relation between cholesterol and phospholipids, which has been considered important in the evolution of atherosclero is Other workers have investigated the relation between the concentration of plasma lipids and the variations in their physicochemical state as studied by ultracentrifugation electrophoresis, etc

Less information is available on the composition of the plasma phospholipid fraction No investigation seems to have been published in which the individual plasma phospholipids have been related to the concentration of other lipids As the phospholipids are considered to be of importance for the stability of the lipid complexes in plasma variation, in the phospholipid composition can be expected to be of great biological importance Some authors (4 6, 7, 9 11 17) studied the amount of individual phospholipids in various lipoprotein fractions and found a higher concentration of lysolecithin and a lower concentration of sphingomyelin in the high density lipoproteins but no notable differences in other phospholipids

The aim of the present study was to investigate in healthy young women the levels of individual plasma phospholipids and their relation to other lipid fractions

Material and methods

During a health survey in which about 800 randomly selected women of different age groups were examined, blood was collected in the fasting state for lipid analyses from 26 young women Three of these women were excluded from the investigation one because of pregnancy and one because she had borne a child less than 3 months earlier The third woman was not pregnant but had on repeated occasions lipid levels in the range found in pregnancy She had been taking a hormonal anticonceptional preparation for two years which was the probable cause of the hyperlipemia (to be published) The remaining 23 women were considered to be healthy on the basis of history physical examination and blood and urine tests 21 women

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TABLE II Correlation coefficients for the relationship between total phospholipid concentration and percentages of individual phospholipid fractions in 23 normal young women r_{AB} = correlation coefficient between A and B Other symbols as in table I None of the correlations are significant

A	B	r_{AB}
Total phospholipids	PE	−0.02
Total phospholipids	Lec	0.17
Total phospholipids	Sph	−0.13
Total phospholipids	LL	0.01

TABLE IV Correlation coefficients for the relationship in 23 normal women between *cholesterol or triglycerides* (mM) and the individual phospholipid fractions expressed as per cent of total phospholipids. r_{AB} = coefficient for correlation between A and B Symbols as in table I

A	B	r_{AB}
Log (10 × triglycerides)	PE	0.13
Log (10 × triglycerides)	Lec	0.03
Log (10 × triglycerides)	Sph	0.04
Log (10 × triglycerides)	LL	−0.16
Cholesterol	PE	−0.02
Cholesterol	Lec	−0.05
Cholesterol	Sph	0.22
Cholesterol	LL	−0.27

Results

Values for individual lipid fractions from all women are given in table I

The correlation coefficients between total phospholipids and the percentages of the different individual phospholipids were found to be not significant (table II)

The correlation coefficients between log (10 × triglycerides) and cholesterol or total phospholipids were not significant but the correlation coefficient between cholesterol and total phospholipids was significant (table III)

The results stated above imply that the percentages of individual phospholipids were not correlated with the cholesterol level The correlation coefficients are given in table IV The correlation coefficients between individual phospholipids and log (10 × triglycerides) were found to be not significant (table IV)

For each of the lipid fractions there was no difference between women who had borne children and those who had not

Lipid values were plotted against the time point of the menstrual cycle when the sample was obtained No evidence of a relationship was seen

TABLE III Correlation coefficients for the relationship between triglycerides cholesterol and total phospholipids in 23 normal women r_{AB} = coefficient for the correlation between A and B P is the significance of the correlation coefficient

A	B	r_{AB}	P
Log (10 triglycerides)	Cholesterol	0.39	0.05 < P < 0.10
Log (10 triglycerides)	Phospholipids	0.39	0.05 < P < 0.10
Cholesterol	Phospholipids	0.83	P < 0.001

TABLE I Plasma lipids in 23 healthy young women PE=phosphatidylethanolamine Lec=lecithin Sph=sphingomyelin LL=lysolecithin S E M =standard error of the mean

	Triglycerides mM	Cholesterol mM	Total phospholipids mM	% of total P lipids				mM			
				PE	Lec	Sph	LL	PE	Lec	Sph	LL
	0 74	4 63	2 85	2 9	69 9	22 1	5 1	0 08	1 99	0 63	0 15
	0 82	5 90	3 32	2 7	68 9	22 2	6 2	0 09	2 29	0 74	0 21
	0 48	4 75	2 99	2 9	69 8	21 0	6 3	0 09	2 09	0 63	0 19
	0 49	3 88	2 34	3 1	66 9	23 8	6 2	0 07	1 56	0 56	0 15
	0 96	5 51	3 35	3 0	67 4	23 0	6 6	0 10	2 26	0 77	0 22
	0 55	4 12	2 77	2 3	68 8	23 0	5 9	0 06	1 90	0 64	0 16
	1 08	5 96	3 25	3 4	70 2	22 1	4 3	0 11	2 28	0 72	0 14
	1 03	6 14	3 94	3 6	68 2	22 4	5 8	0 14	2 69	0 88	0 23
	0 91	4 56	3 21	2 7	71 2	20 3	5 8	0 09	2 28	0 65	0 19
	0 63	5 52	3 58	2 0	70 9	20 1	7 0	0 07	2 54	0 72	0 25
	0 76	4 93	2 82	2 8	70 7	22 6	3 9	0 08	1 99	0 64	0 11
	0 78	5 21	3 16	2 6	71 2	19 5	6 8	0 08	2 25	0 62	0 21
	0 66	7 21	3 66	2 5	67 1	25 5	4 9	0 09	2 46	0 93	0 18
	0 54	5 08	3 03	3 2	68 4	23 0	5 4	0 10	2 07	0 70	0 16
	0 81	5 19	2 89	3 0	67 9	23 5	5 6	0 09	1 96	0 68	0 16
	0 86	5 41	3 14	2 7	69 9	23 6	3 8	0 09	2 19	0 74	0 12
	0 74	5 58	3 41	2 4	70 9	21 5	5 1	0 08	2 42	0 73	0 18
	1 41	5 82	3 36	2 7	69 5	21 9	5 9	0 09	2 33	0 74	0 20
	0 72	5 61	2 89	2 7	69 9	23 2	4 2	0 08	2 02	0 67	0 12
	0 57	4 73	2 85	3 0	69 5	21 2	6 4	0 09	1 98	0 60	0 18
	0 77	6 85	3 68	2 6	68 7	23 5	5 2	0 10	2 53	0 87	0 19
	0 51	5 81	3 56	3 1	72 1	19 7	5 1	0 11	2 56	0 70	0 18
	0 81	6 07	3 66	3 1	70 8	21 2	5 0	0 11	2 59	0 78	0 18
Mean	0 77	5 41	3 21	2 8	69 5	22 2	5 5	0 09	2 23	0 71	0 18
S E M	0 218	0 166	0 079	0 08	0 30	0 31	0 19	0 003	0 057	0 019	0 007

were 23 years old 2 were 30 years 15 women were nulliparae, 6 I parae and 2 women were II parae

Methods for determination of total and individual phospholipids, cholesterol and triglycerides were the same as described previously (14, 15) Each determination represents the mean of four values except in a few instances where only duplicate determinations were performed

Statistical calculations were made according to Snedecor (12) In the statistical analysis of the relationship between different lipid fractions a linear model was assumed in the range of observed values In this analysis the individual phospholipids were expressed as per cent of total phospholipids For triglycerides calculations were made with logarithmic conversion, calculated on (10 × molar concentration) to avoid negative values The relationship between different lipid fractions was studied by the calculation of correlation coefficients To test the null hypothesis of no correlation the t test was used All tests of significance were performed at the 5 per cent level

composition of the phospholipid fraction is independent of the levels of cholesterol and triglycerides Some degree of correlation might have been expected as differences have been reported between different lipoprotein fractions, with regard both to phospholipid composition and to contents of cholesterol and triglycerides The negative finding is probably due mainly to the small differences in phospholipid composition between the lipoprotein fractions

The failure to detect a relationship between lipid concentrations and the stage of the menstrual cycle in this group probably means only that inter individual variations are large in comparison with the variations within the menstrual cycle which seem to be well substantiated in the case of cholesterol and phospholipids (1 10) For a more exact evaluation of changes in phospholipid composition and triglycerides during the cycle serial studies must be performed

The main finding in this study was that in normal young women the composition of the plasma phospholipids is independent of the total lipid level and that the composition is remarkably constant It is possible that this precise balance is in some way optimal for the action of this fraction whether for transport mechanisms for effects on blood coagulation or for other actions which the phospholipids may have in blood

Summary

Plasma lipids including individual phospholipids were determined in 23 healthy young women

The phospholipid composition was found to be remarkably constant and independent of the levels of total phospholipids, cholesterol or triglycerides

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Discussion

The women investigated in this study are considered to be *representative* of *healthy* young women in this area of Sweden. As plasma lipid values are *influenced* by many factors, e. g. age, diet, seasonal variations, methodological differences etc., *slight* variations are to be expected from study to study. However, *the values reported here for cholesterol*, total phospholipids and triglycerides are in general agreement with those found by other Swedish investigators (3, *8, 13*).

The values for the percentage of the different phospholipids agree closely with those found previously in a smaller series of healthy young women (15). The values for lysolecithin thus are still slightly lower than those found in the literature. Recently Wagener et al. (16) presented normal values which gave a mean lysolecithin value of 9.4 per cent with rather large variations. The authors used serum and considered the variations in lysolecithin to be partly due to formation of lysolecithin *in vitro*. It seems important to perform immediate extraction of blood in order to get as exact values for lysolecithin as possible.

As has been found previously by Carlson (2) for triglyceride values in men and by Feldman et al. (5) for women there was also in this material a skew distribution. The statistic g_1, the coefficient of skewness, was 1.13 and its standard deviation, s_g, 0.48. On testing the null hypothesis of symmetry, the coefficient was found significant at the 5 per cent level. The logarithmic conversion was then tried and the null hypothesis of symmetry in the logarithmic values was tested and accepted. Thus in this study log (10 × molar concentration) was used in calculations on the relationship between triglycerides and other lipid fractions. For cholesterol and total phospholipids a check for symmetry was also done and no significant asymmetry was discovered.

The observation that there was no correlation between percentages of phospholipids and total phospholipid values indicates that the composition of the phospholipid fraction is independent of the total level. From this result it follows that there is a correlation between the absolute values of individual phospholipids and the total phospholipid levels.

There was a close correlation between total phospholipids and cholesterol, while the correlation between log triglycerides and either cholesterol or phospholipids did not quite reach the 5 per cent significance level. The close relationship between phospholipids and cholesterol has been reported in many studies. While some authors have found significant correlations between triglycerides and the other two fractions (2, 5), the correlations have been less close than between cholesterol and phospholipids. In a material of 99 women of all ages Feldman et al. (5) found a correlation coefficient of 0.37 between cholesterol and log triglycerides which in that material was highly significant. This coefficient is similar to that found in the present study of a smaller material (0.39).

The lack of correlation between individual phospholipids and cholesterol or log triglycerides follows partly from the relationship between these fractions and total phospholipids. This shows that the

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Plasma Cholesterol Esterification and Lysolecithin Production in Vitro during Pregnancy Hyperlipemia

By

Alvar Svanborg and Olle Vikrot

In 1935 Sperry (13) observed that when serum or plasma is stored at 37° C for three days a net esterification of cholesterol occurs Glomset et al (7) found that the fatty acids in the cholesterol esters so produced were transferred largely from lecithin which was thus transformed into lysolecithin It was suggested (5 6) that this mechanism (fatty acid transferase reaction) may be largely responsible also for the formation of lysolecithin *in vivo*

In pregnancy there is a characteristic decrease of plasma lysolecithin (14, 15) and changes in the transferring enzyme activity might be of etiological importance for this decrease The aim of the present investigation was to study *in vitro* the cholesterol esterification and lysolecithin formation in plasma from pregnant women

Material and methods

Blood was drawn from 5 women aged 20—33 years with duration of pregnancy 20—39 weeks The control group consisted of 5 healthy women aged 20—30 years

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Heparin plasma was extracted before and after incubation for 6 hours at 37 C. Methods for extraction and for phospholipid analyses have been described previously (15) Unesterified cholesterol was determined by the method of Crawford (1) Statistical calculations were made according to methods presented by Snedecor (12)

Results

The results are summarized in table I The pregnant women had a low lysolecithin level (1 7—2 5 per cent of the total phospholipids) while the control group had a normal percentage of lysolecithin (5 0—7 4) (8)

The changes in free cholesterol, lecithin and lysolecithin after incubation are given both as absolute changes and as per cent of the initial values There were no significant differences between pregnant and non pregnant groups, except that the percentage increase in lysolecithin was higher in the pregnant group, due to the lower initial values

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is increased acylation with formation of lecithin, a reaction which occurs in e g liver (9) and erythrocytes (10, 11)

Summary

The fatty acid transferase activity was studied *in vitro* in plasma from 5 pregnant and 5 non pregnant women

After incubation of the plasma at 37 C for 6 hours the decrease of free cholesterol and lecithin and the increase of lysolec ithin were similar in both groups

These observations indicate that the low level of plasma lysolecithin during pregnancy is not due to a change in the fatty acid transferase activity

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TABLE I Changes in free cholesterol, lecithin and lysolecithin after incubation of heparin plasma for 6 hours at 37 C n = number of women S E M = standard error of the mean

		Free cholesterol			Lecithin			Lysolecithin		
			Decrease			Decrease			Increase	
		Initial value mM	mM	% of initial value	Initial value mM	mM	% of initial value	Initial value mM	mM	% of initial value
Pregnant	Mean	1 93	0 29	16 0	2 88	0 28	9 6	0 09	0 23	285
n = 5	S E M	0 07	0 04	1 3	0 03	0 02	0 6	0 004	0 01	17
Non pregnant	Mean	1 59	0 31	17 6	2 18	0 26	12 0	0 18	0 24	135
n = 5	S E M	0 13	0 03	1 5	0 19	0 04	1 4	0 010	0 01	7

Discussion

The decreases of free cholesterol and of lecithin were similar and the rate of cholesterol esterification in this study was of the same order of magnitude as that found by Glomset (5) He reported an initial rate of cholesterol esterification of 0 11 mMol/L/hour, but the rate was linear only for the first few hours The increase of lysolecithin was somewhat lower than the decrease of lecithin in the present study, which is also in accordance with Glomset's findings According to Etienne and Polonovski this is due probably to a further breakdown of lysolecithin to glycerylphosphorylcholine (2, 3, 4)

The incubation medium was different in the two groups The "substrate" level, i e the concentration of lecithin and free cholesterol, is higher in the pregnant group and the concentration of the "end products" is also different, viz higher level of cholesterol esters and lower level of lysolecithin Thus, the present observations do not allow any definite conclusions concerning the transferase enzyme activity *per se*, but indicate that the net effect of this process is of the same order of magnitude in pregnant and non pregnant women

The rate of the breakdown of lysolecithin to glycerylphosphorylcholine was not studied in these experiments, but the fact that the changes in cholesterol, lecithin and lysolecithin were similar in the two groups, gives no support for a difference

In the discussion concerning the mechanisms involved in the regulation of the lysolecithin level in plasma these observations might be of importance The very low level of lysolecithin in pregnancy and the normal net transferase activity indicate that at least in pregnancy, the marked changes in the lysolecithin concentration are due to mechanisms other than the transferase activity One such possible mechanism

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From the Medical Department (Head H G Dedichen, M D), Lillehammer Fylkessykehus, Lillehammer, Norway

Spontaneous Reversion of Ventricular Fibrillation Complicating Acute Myocardial Infarction

By

Gunnar Alm Rosland

The pathophysiology of ventricular fibrillation is rather complex (1, 9), and consequently an evaluation of the various factors may not be easy However ventricular fibrillation (v f) caused by severe heart damage such as acute myocardial infarction is more fixed and more resistent to therapy than v f in certain reflex cardiac arrests The latter may nearly always be reversed by cardiac massage alone (9)

According to recent publications (3 8) cardiac arrest in acute myocardial infarction is more frequently caused by v f than by ventricular asystole In spite of this very few cases with spontaneous reversion of v f in acute myocardial infarction have been reported

In 1961 Jude et al (7) found that 30 % of cardiac arrests in their series of 138 (118 patients) occurred with v f which necessitated electrical defibrillation in all instances Recently Julian et al (8) in a prospective study with continuous ECG monitoring of 100 consecutive unselected patients with acute myocardial infarction, observed attacks of v f in 10 patients, but spontaneous reversion occurred only once This one included, there are reports in the literature of seven cases (2, 4, 5 6, 8 10, 11) suffering acute myocardial infarction complicated by v f which reverted without electrical defibrillation In one case (5) the attack seemed to be provoked by acute anxiety Apart from two cases (4 10) the patients were treated with heart massage during the attacks The reported cases comprise 3 females 3 males and one whose sex is not stated Five cases whose age is reported have an average age of about 62 years (50—76)

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Our case

A 66-year-old woman was admitted to the medical department of Lillehammer County Hospital 6 5 1963 with a diagnosis of acute myocardial infarction

In July 1962 she experienced an attack of precordial pain lasting for about one hour She felt weak for a couple of days afterwards but soon resumed her normal activities with no discomfort She did not consult a

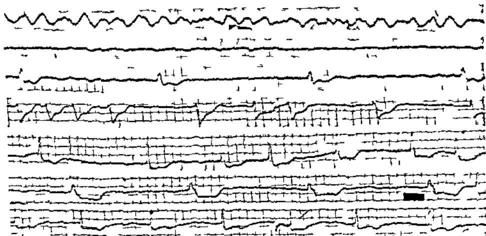


Fig 2 All tracings from lead I
1 line At 12 46 p m (about 18 min after cardiac arrest) showing v f
2 line At 12 55 p m ventricular asystole (Periods with electrical activity representing neg P waves?)
3 line At about 12 58 p m spontaneous idioventricular rhythm rate 32 None or irregular atrial activity
4 line At 1 15 a m A repetitive type of ventr tachycardia superimposed on a slow idioventricular rhythm Two ventricular captures are seen no sign apart from these of atrial activity
5 line At 1 20 a m Possibly atrial fibrillation
6 line At 1 30 a m Complete A V dissociation regular ectopic rhythm One ventricular capture
7 line At 1 45 a m Second degree heart block (2 1)

About 10 minutes after regaining spontaneous heart action the patient was conscious though somnolent and responded adequately when spoken to No signs of cerebral complications were found (We did not record an EEG)

Treatment after resuscitation (no treatment of acidosis was undertaken after recovery) She received 120 mg Phenindandione and procaine amide 0 5 g × 4 At 8 30 a m she had a tracheotomy

After resuscitation the patient was oliguric with 100 ml of urine during the first 24 hours

At 12 40 a m 8 5 63 she developed a fulminating pulmonary oedema and died

Autopsy

The body of a 66 year-old woman slightly overweight was examined A coronary atheromatosis was found of degree III—IV with an almost total atheromatous occlusion of the lumina in the descending branch and in the circumflex branch of the left coronary artery In the right coronary artery 4 cm distal to the ostium the lumen was totally occluded by a fresh thrombus Another fresh thrombus had been formed 1 cm distal to the first one Correspondingly there was a recent haemorrhagic myocardial infarction occupying the posterior one third of the interventricular septum and most of the posterior wall of the left ventricle The lungs showed oedema

The kidneys appeared normal on macroscopical examination

Comment

V f in acute myocardial infarction is thought to be more frequent in a relatively young age group, the average age being about 50 years (8) Thus *there will be* a great preponderance of male patients Julian et al (8) report only 5 females in a total of 56 patients

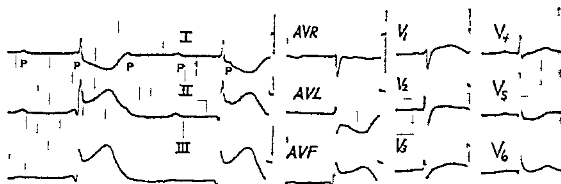


Fig 1 12 lead ECG tracings shortly after admission showing a transmural posterior wall infarction and complete A V dissociation Ventricular rate 47 per minute

physician Apart from this she had suffered no serious illness before

On the 3rd of May 1963, at bedtime, she had a new attack of precordial pain lasting for about thirty minutes She felt weak, dizzy and nauseated On May 4th and 5th she had several attacks of the same type, but of short duration On May 6th she felt perfectly well and spent the evening at a dinnerparty Soon after dinner about 11 o'clock p m she felt unwell and shortly afterwards felt a strong precordial pain After a couple of minutes she collapsed and remained unconscious for one to two minutes She recovered spontaneously and was brought directly to the hospital by car

On admission to the hospital she was in pre shock Oxygen was administered through a nasal catheter and she was given 1 ctg of morphine c atropini i m followed some minutes later by 0 4 ml of Aramine 1 % i v Her condition improved and the blood pressure increased from 80 60 to 145 85

A 12 lead ECG showed signs of an acute transmural myocardial infarction of the posterior wall and complete A V block with a ventricular rate of 47 per minute (fig 1) Haemoglobin 14 4 g % erythrocyte sedimentation rate 10 mm, leucocyte count 11,300, PP (Owren & Aas) 90 % S GOT 200 units 7 hours after admission

The initial examination was ended about 12 15 p m 7 5 63, and the patient was brought to the ward No further treatment had yet been initiated when at about 12 25 p m she had cardiac arrest Some 2 minutes later closed chest cardiac massage and mouth to mouth respiration was started Shortly afterwards 0 4 ml of 1 % epinephrine was injected intracardially, apparently with no effect 15 minutes after the cardiac arrest had taken place, the patient was intubated with an endotracheal tube and ventilated with pure oxygen by means of a Rubens bag There was little or no dilatation of the pupils and no cyanosis Following each thoracic compression arterial pulse could clearly be felt in the groins By this time she regained spontaneous respiration though inadequate The next ECG-tracings were obtained at 12 46 p m They showed a state of ventricular fibrillation (fig 2) I v injection of procaine amide was prepared However before this was given, ECG showed conversion into ventricular asystole at 12 55 pm (ECG-tracings with intervals show a total of 80 seconds with v f) While preparing to give an injection of epinephrine spontaneous idioventricular cardiac action recurred at 12 56 pm The further development is seen in fig 2 the last strip, obtained at 1 45 a m showing heart block of second degree

With regained, regular cardiac action and a ventricular rate of about 80 per minute systolic blood pressure was below 50 and an i v drip of Nor-exadrine was initiated and continued uninterrupted

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From the Department of Internal Medicine and the Clinic of Odontology Blegdams Hospital, Copenhagen Denmark

Tongue Biopsies in Various Clinical Conditions

By

HENNING JENSEN, E HJORTING HANSEN and KAJ KJERULF

It is generally accepted that an overall medical examination should include an examination of the patient's tongue While numerous clinical descriptions of the tongue in diseased persons are available only very few studies are based upon biopsies of the tongue itself

Oral manifestations of internal medical diseases are described during the later years of Thoma (21), McCarthy and McCarthy (10) Osbourn (15) Thoma (22) Epsteen (5) Rushton and Cooke (17) Kaplan (8) and Balogh and Lelkes (2) Frantzell et al (6) published their observations in a clinical and photographic study of the tongue in 1 300 patients and 200 healthy persons

The histopathology in the tongue has so far mainly been based upon histological studies on material obtained at autopsy Pagel (16) presented such findings Studies involving tongue biopsies in living persons were described by Stein and Gold (18) in cancer patients and by Stein and Spencer also (19) in 3 patients with extreme malnutrition In 1958 Taft et al (20) reported their experiences when they performed 50 tongue biopsies with a rigid stomach tube for suction biopsy A similar technique was applied by McLean et al (11) before and after treatment with iron preparations in 14 patients with simple sideropenic anaemia

In the study presented here the authors have aimed at clarifying the following question Are the histopathological alterations of the tongue in various prolonged diseases qualitatively of the same type or are they specific to particular groups of internal medical disorders?"

Technique

The technique applied here permitting repeated biopsies of the tongue is described in the preceding article in this journal by Hjorting Hansen Jensen and Kjerulf (7)

Inspection of the tongue The normal tongue the dorsum has a velvety whitish appearance *due to the presence of 1—3 mm long horny* filiform papillae The sparsely scattered fungiform papillae usually hidden by the filiform papillae are red round and smooth (fig 1)

Atrophy of the mucous membrane of the tongue starts at the margins and at the tip of the tongue The filiform papillae are the first to disappear The area becomes shining and

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with v f In contrast to this the referred cases (2, 4, 5, 6, 8, 10, 11) with *transient* v f in acute myocardial infarction show approximately an equal number of males/females

Complete A-V block secondary to acute myocardial infarction is seen almost exclusively in connexion with occluded right coronary artery and ischaemic affection of the upper, posterior part of the interventricular septum The prognosis is poor when this rhythmic disorder complicates an acute myocardial infarction The natural mortality is believed to be 60—100 % (1), but the mortality might be reduced by adequate therapeutic means (8) Some authors advocate the use of steroids presuming that inflammatory reaction surrounding the infarcted area may involve the A-V node (1)

We considered it necessary to give our patient a continuous i v drip with Nor-exadrine to maintain a satisfactory blood pressure during the 24 hours which elapsed before death, though aware that the use of pressor amines would increase the systolic load of the left ventricle The fatal pulmonary oedema may have been elicited by Nor-exadrine

Our case might show that it is worth while to carry on with the means of resuscitation available, even if the facilities for further treatment are poor

Summary

A 66-year-old female suffered an acute, posterior wall myocardial infarction complicated with heart block of third degree The patient later developed cardiac arrest with ventricular fibrillation Spontaneous recovery of cardiac action took place with no other treatment than closed chest cardiac massage and artificial venulation

A brief review is given of the cases previously reported in the literature.

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Fig 1 Normal tongue showing numerous filiform papillae



Fig 2 Tongue with advanced mucosal papillary atrophy

slightly more red Finally the tongue may become smooth and shining due to the complete disappearance of the filiform and fungiform papillae The colour may vary from pale to red even fiery red often combined with fissuring and lobulation (fig 2)

Histopathology in tongue mucosal atrophy The normal tongue is microscopically characterized by the presence of papillae fungiform with a regular connective tissue stalk the filiform as pure epithelial formations (fig 3) The superficial layers of the epithelium are matured in a special way morphologically resembling parakeratosis however not staining like keratin Slight atrophy results in a flattening of the filiform papillae without causing any changes of the fungiform papillae Progressive atrophy causes gradual disappearance of the filiform as well as fungiform papillae resulting in a nearly complete straight lined surface of the epithelium combined with a regular parakeratosis (fig 4) The disappearance of the papillae may be associated with a marked atrophy of the entire epithelial layer

Selection of patients The following criteria were considered before a biopsy was made 1 The clinical diagnosis should be beyond doubt 2 The clinical condition should be dominated by one disease Slight arteriosclerosis according to our present observations does not modify the tongue structure and does not cause atrophy 3 Specific therapy, particularly with iron preparations antibiotics or antiinflammatory steroids and vitamins should not be in use except in cases where the effect of such therapy on the tongue was to be studied Medicine such as phenthiazine chloramphenicol etc had to be withdrawn at least two weeks before the biopsy was performed

According to these conditions 70 patients 40 women and 30 men were examined Their age was predominantly between 60—80 years with an average of 69 years the youngest being 25 years old the oldest 94 years old

The patients were grouped as follows blood diseases table I gastrointestinal diseases table II metabolic disorders (diabetes mellitus) table III diseases of connective



Fig 3 Histological pattern of normal tongue showing filiform papillae



Fig 4 Histological pattern in tongue with advanced papillary atrophy

tissue table IV lung and heart diseases, table V, medical abuse and central nerve disturbances table VI renal disorders and controls were not recorded in the tables The control group comprised 7 persons aged between 60 and 70 years without any known major disease particularly without anaemia, subnormal serum iron concentration or gastric hypochlorhydria while a moderate non symptomatic arteriosclerosis was allowed

Findings

Blood diseases This group includes 16 patients (22 biopsies) table I

The most striking observation was made in 6 patients with sideropenic anaemia 3 of these were examined before and after treatment with oral ferrous tartrate Patient No 4 presented a normalization of the haemoglobin values and tongue epithelium after 6 weeks treatment Patient No 5 presented a normal surface of the tongue at biopsy already 16 days after therapy with oral ferrous tartrate had been initiated This patient was generally improved although still anaemic In patient No 6 the treatment with ferrous tartrate was discontinued after one day and no response occurred either in the blood or in the histological pattern of the tongue

One of the patients with low serum iron was a 25 year old woman (No 3), haemoglobin was 7 6 g %, serum iron concentration 15 μg % responding well to ordinary oral iron therapy In this case of simple bleeding anaemia the tongue appeared normal macroscopically as well as histologically

Three patients (Nos 7 8 and 9) with pernicious anaemia all had typical tongue atrophy at biopsy before treatment with vitamin B_{12} injections was initiated A second biopsy was performed after 2 4 and 5 weeks respectively Regenerative processes of the papillae could be demonstrated after 2 weeks and were complete after 4—5 weeks treatment in these patients

Three other patients with pernicious anaemia who had been treated constantly and according to present clinical routine had pronounced tongue atrophy although serum vitamin B_{12} concentration was subnormal in only one of these



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TABLE II Mucosal atrophy of the tongue in various disorders of gastrointestinal function

	Pat. no	Hb (g %)	Serum Iron (μg %)	Serum Transferrin (μg %)	Gastric HCl	Tongue biopsy histopathology
Diverticulum of oesophagus	1	13 5	57	219	−	Normal
Psychosomatic colon neurosis	2	13 8			+	Normal
	3	14 3			−	Normal
	4	12 0	71	241	+	Normal
Chronic gastritis hypochlorhydria	5	14 5	23	229	−	Atrophy
	6	11 4	69	380	−	Normal
	7	13 9				Normal
Sequels of gastric resection	8	11 3	17	266	−	Normal
	9	11 1	23	360	−	Atrophy
	10	13 6				Atrophy
Carcinoma of the ventricle	11	8 2	51	188	−	Atrophy
Regional enteritis	12	12 1	52	383	+	Atrophy
Intestinal malabsorption syndrome	13	12 9	95	204	−	Atrophy
Chronic pancreatitis	14	15 2	325	275	+	Normal
	15	14 5	52	344		Atrophy

tongue except in one case complicated with sideropenia

Mucosal atrophy was also found in cases of chronic pancreatitis with border line low serum iron concentration Patient No 12 with regional enteritis diarrhoea and considerable weight reduction did not respond to treatment with antiinflammatory glucocorticosteroids tetracycline ferrous tartrate and vitamins and a second biopsy showed unaltered tongue atrophy at microscopy

Metabolic or endocrine disorders This group includes 8 patients with diabetes mellitus and 1 with Addison s disease table III

Five cases of diabetes had normal tongue mucosa at histological examination of the biopsy material while 3 others showed atrophy These alterations could not be ascribed to lack of iron or vitamin C B_1 B_{12} folic acid except for one case which showed an abnormally low content of ascorbic acid in the serum there was however no other symptoms indicating vitamin deficiency It is noteworthy that in other patients low concentrations of vitamin C in serum were not associated with tongue mucosal atrophy The occurrence of tongue changes in these diabetic patients could not be referred either to the duration or the regulation period of the diabetes

TABLE I Mucosal atrophy of the tongue in patients with various disorders of blood formation

	Pat no	Hb (g %)	Serum Iron (μg %)	Serum Transferrin (μg %)	Serum Vitamin B_{12} (pg/l)	Serum Vitamin C (mg %)	Gastric HCl	Tongue biopsy histopathology
Anaemia, iron deficiency	1	5.2	73	388	480	7.4		Atrophy
	2	9.9	29	312	244	8.0	–	Atrophy
	3	7.6	15	316			–	Normal
	4	6.5	35	385			+	Atrophy
		14.4						Normal
	5	8.4	11	296	280		–	Atrophy
		8.4	265	408		0.5		Normal
	6	8.8	68	470		0.2	–	Atrophy
		9.0						Atrophy
Pernicious anaemia before and after vitamin B_{12} injections	7	7.7	270	338	180		–	Atrophy
		9.7						Normal
	8	7.7			50		–	Atrophy
		11.2					–	Normal
	9	11.0	157	265			–	Atrophy
		15.1						Normal
	10	8.4	155	244			–	Atrophy
Pernicious anaemia treated	11	13.0	73	233	100		–	Atrophy
	12	16.2	108	394	430		–	Atrophy
	13	16.1	68	320	460	12.0	–	Atrophy
Aplastic anaemia	14	5.1	195	221			–	Atrophy
Leucaemia chr lymphatic	15	7.8						Atrophy
Generalized myelomatosis	16	8.5	73	160			–	Atrophy

and serum iron concentration above lowest normal level In blood dyscrasias aplastic anaemia, chronic lymphatic leucaemia and generalized myelomatosis involving the nutritional state and condition of the patient a pronounced mucosal atrophy was found macroscopically as well as microscopically

Gastrointestinal diseases This group include 16 patients, table II

Mucosal atrophy of the tongue was found within such diseases that may affect the nutritional state of the diseased person i e in this series postresectional syndrome, carcinoma of the ventricle regional enteritis and intestinal malabsorption

The group psychosomatic neurosis of the colon and gastritis even with hypochlorhydria was not found to be associated with mucosal atrophy of the

TABLE II Mucosal atrophy of the tongue in various disorders of gastrointestinal function

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TABLE III Mucosal atrophy of the tongue in 8 patients with diabetes mellitus and one with Addisons disease

	Pat no	Hb (g %)	Serum Iron (µg %)	Serum Transferrin (µg%)	Gastric HCl	Diabetic retinopathy	Tongue biopsy histopathology
Diabetes mellitus	1	14 5	104	269	+	Severe	Normal
	2	13 5					Atrophy
	3	13 4	79	238	+	Moderate	Normal
	4	12 5	80	116	–		Normal
	5	13 7	136	303		None	Atrophy
	6	15 0	55	212	+	Moderate	Normal
	7	14 4	70	262	+	None	Atrophy
		14 3	77	276	+	None	Atrophy
	8	12 5				Severe	Normal
Addison's disease	9	12 6	44	328			Atrophy

Patient No 7 with diabetes mellitus, who had mucosal atrophy of the tongue, was poorly regulated at the time for the first biopsy, the histopathology was, however, unaltered at a second biopsy after a period of 4 weeks with adequate diet and insulin and considerable improvement of the general condition of the patient

One patient with Addison's disease treated with cortisone and aldosterone had mucosal atrophy of the tongue for unknown reasons

Diseases of connective tissue This group comprises 5 patients with autoimmune diseases, table IV

One patient with untreated, moderate, disseminated lupus erythematosus had normal tongue mucosa although the haemoglobin value was relatively low

In the first mentioned case with Sjogren's syndrome treatment with antiinflammatory steroids was not used in the interval between biopsy numbers one and two

The patient with fully developed dermatomyositis had low serum iron and anaemia both refractory to treatment with ferrous tartrate orally Treatment with prednisone improved the general condition of the patient in few days, haemoglobin became normal without specific administration of iron preparations and a second biopsy was made, when steroid treatment had lasted only 3 weeks The tongue had already changed from the atrophic to normal pattern macroscopically as well as histologically

Patients with lung and heart diseases, table IV Among the 6 patients in this group only one patient of those with chronic relapsing bronchitis presented tongue papillary atrophy, this patient however had also gastric hypochlorhydria This series did not involve the study of severe

TABLE IV Mucosal atrophy of the tongue in patients with chronic bronchitis vascular diseases and auto immune connective tissue diseases

	Hb (g %)	Serum Iron (γ g %)	Serum Transferrin (μg %)	Gastric HCl	Tongue biopsy histopathology
Chronic bronchitis	14 4				Normal
	15 0				Normal
	14 4	89	236		Normal
	13 8			–	Atrophy
Pneumonia pleural effusion	12 9	41	303		Normal
Arteriosclerotic heart disease	13 9	74	249		Normal
Lupus erythematosus disseminatus	9 8	45	373	+	Normal
Keratoconjunctivitis sicca Sjogren	12 8	73	336	+	Atrophy
	10 8				Atrophy
	11 1	23	132		Atrophy
Dermatomyositis	8 8	37	96	+	Atrophy
	13 5				Normal
Rheumatoid arthritis (chronic)	14 1			+	Normal

chronic respiratory insufficiency with constantly decreased arterial blood oxygen tension and carbon dioxide retention

Patients with malnutrition and avitaminosis table I In a case of severe prolonged malnutrition with low serum ascorbic acid concentration no papillary atrophy of the tongue could be demonstrated One patient with malnutrition anaemia slight sideropenia and low serum vitamin B_1 responded to supplements of vitamin B_1 alone (and ordinary daily food) with an increase in haemoglobin and a return to normal of the tongue mucosa with regeneration of the papillae

Liver and bile diseases Even pronounced liver cirrhosis table V might be associated with a normal i e non atrophic tongue mucosa A case of alcoholic cirrhosis of the liver in a patient with a poor food intake had low values for serum iron and presented a pronounced pattern of mucosal atrophy of the tongue This was also found in a case of carcinoma of the gall bladder with metastases In a case of chronic relapsing cholecystitis which was not complicated with sideropenia and not treated with antibiotics no evident cause for the existing tongue atrophy could be demonstrated

Patients with abnormally high intake of medicine and patients with central nerve disturbances table VI The first patient in table VI had prolonged abuse of various types of medicine, barbiturates phenacetin and sedatives including phenthia

TABLE V Mucosal atrophy of the tongue in patients with liver and bile diseases and in vitamin deficiency states

	Hb (g %)	Serum: Iron (μg %)	Serum: Transferrin (μg %)	Serum: Vitamin B_{12} (pg/ml)	Serum: Folic acid (μg/ml)	Vitamin C (mg %)	Urine vitamin B_1 (μg per 24 hours)	Gastric HCl	Tongue biopsy histopathology
Vitamin C deficiency	13 8	77	159			0 13		+	Normal
Vitamin B_1 deficiency	6 6	27	432	250	0 008	0 44	[1] 320	+	Atrophy
	12 4	28	580		0 008	0 83	[1] 2 480		Normal
Liver cirrhosis non alcoholic	11 4								Normal
Liver cirrhosis chronic alcoholic	11 0	29	343	420	0 010	0 31		–	Atrophy
Cholecystitis	14 4	133	405	300	0 006	0 12		–	Atrophy
Carcinoma vesicae felleae cum metastases	12 7	13	105	790	0 006				Atrophy

[1] Vitamin B_1 loading test excretion before and after therapeutic administration of thiamine.

TABLE VI Mucosal atrophy of the tongue in patients with abuse of medicine and in cerebral thrombosis involving hemiparesis of the tongue

	Hb (g %)	Serum: Iron (μg %)	Serum: Transferrin (μg %)	Gastric HCl	Tongue biopsy histopathology
Abuse of medicine phenacetine etc	13 0	60	250	+	Atrophy
Stomatitis *medicamentosa bismuth* subcarbonate	14 6				Atrophy
Cerebral thrombosis hemiparesis	13 6	58	328	–	Normal
	11 6	51	203	+	Normal

zines resulting in a poor general condition and a pronounced mucosal atrophy of the tongue

The second patient, with chronic duodenal ulcer, had for years daily taken bismuth subcarbonate orally

t inspection of the oral cavity all mucosal surfaces were markedly red and scrapings from the patient s dentures contained bismuth on chemical analysis

In order to demonstrate an eventual mucosal change from lack of nerve supply to the oral cavity 2 subsequent biopsies were made in 2 patients with acute vascular insufficiency of the brain due to cerebral vascular thrombosis complicated with unilateral tongue and soft palate muscle paralysis The interval was 4 weeks and no atrophy which might have been taken as a consequence of the impairment of motility and trophic nerves of the tongue was observed

Patients with renal diseases These two patients one with chronic pyelonephritis and one with renal amyloidosis verified through kidney biopsy had both pronounced tongue papillary atrophy The tongue tissue however did not contain any amyloid substance on histological examination

Control group These seven persons had all normal papillary pattern at inspection of the tongue and on histological examination

Throughout this study a very good correlation with regard to papillary atrophy was found between the tongue mucosal surface as estimated by inspection and its eventual papillary atrophy as estimated histologically in the biopsy material

Discussion

In order to evaluate the aetiology of mucosal atrophy of the tongue those patients who had one or more biopsies performed were grouped within common clinical entities Among the biopsies performed before any treatment in 70 various patients only 33 were normal, the pathological biopsies within completely different medical diseases all showed major or less papillary atrophy of the tongue mucosa, and no other alterations were found Although several aetiologies had to be considered, they might all end up with the same degenerative tissue change Thus tongue mucosal atrophy was very likely to be the result of an impairment of cell metabolism

The following factors might evidently be of some significance low haemoglobin concentration, sideropenia gastric hypochlorhydria and malnutrition

It is well known that prolonged and pronounced anaemia may be associated with macroscopically evident mucosal atrophy of the tongue with total loss of of the tongue papillae In this study 18 patients had for various reasons haemoglobin values below 9 g % 15 of these had mucosal atrophy at biopsy while 13 patients among 30, who had haemoglobin values within normal limits, still presented tongue atrophy Thus the papillary atrophy may very well exist also in non anaemic persons

Sideropenia manifesting as prolonged subnormal serum iron concentration has been considered along with anaemia Serum iron concentration was determined in 61 of the 70 persons studied Values below 75 μg % and 50 μg % were found in 41 and in 17 patients respectively among these papillary atrophy of the tongue was found in 24 and in 11 patients respectively

Anaemia and/or sideropenia or the underlying disturbance should exist for

some time before the mucosal atrophy becomes evident According to Balogh and Lelkes (2) mucosal atrophy does not develop with age as such In the present study no evidence of mucosal atrophy of the tongue as a sequel to age or arteriosclerosis was found

The present study is well in accordance with McLean et al (11) and Darby (4), who described the regeneration of tongue papillae as a very sensitive indication for the response to the therapeutic administration of iron preparations in sideropenic anaemia

Waldenstrom (23) and Frantzell et al (6) drew attention to the fact that iron deficiency may interfere not only with the formation of haemoglobin but also the iron containing cell oxydation enzymes They concluded that the condition of the tongue papillae might be "a mirror of cell oxidation" This view is well in accordance with the fact that lack of other factors may interfere with the condition of the tongue papillae particularly nicotinic acid, vitamin B_1, folic acid, thiamine and possibly riboflavin, while the significance of vitamin C, D and A and some biogenic amino acids, tryptophane for example, may still be discussed

Several authors have pointed out that tongue atrophy in pernicious anaemia and pellagra cannot be distinguished macroscopically Mansohn Bahr added sprue, nutritional anaemia and ideopathic steatorrhoea as possible causes of tongue atrophy, and Thoma (21) described pellagra, beri beri and ariboflavinosis with tongue mucosal alterations

In the present study one case of psychogenic malnutrition with a pronounced vitamin B_1 retention reacted promptly to vitamin B_1 therapy with clinical improvement and normalization of the atrophic tongue papillary pattern The regeneration was evident before a complicating low serum iron concentration had been altered significantly It should be mentioned, however, that the appetite of such a patient may increase considerably during the recovery period, and the intake of proteins, (amino acids and iron) may consequently be higher than previously in the period of anorexia

In most cases the state of deficiency involves the lack of several factors including B vitamins and iron Middleton (12) points out that tongue mucosal atrophy in sideropenia is quite similar to that in pernicious anaemia The existence of a complicating vitamin B deficiency should be considered, Darby (4), and therapy in such cases should involve the relevant B vitamins and iron to induce a complete regeneration of the glossitis, Afonsky (1) In this study isolated vitamin C deficiency in one case was not associated with mucosal atrophy of the tongue

The frequency of atrophic glossitis in pernicious anaemia as mentioned in the literature is not yet fixed Cox (3) investigated 548 patients and found only 56 % with tongue atrophy Three of our patients with pernicious anaemia had still tongue changes in spite of therapy Neither lack of iron nor folic acid seemed to offer an explanation Their therapeutic supply of vitamin B_{12} may however, have been insufficient contrary to the information, given by the patients Folic acid deficiency, which according to Mollin (13) is so common in elderly

people in England, seems to be much more rare in Denmark, and during this study subnormal folic acid blood levels of clinical significance were not found in any of our patients, 30 in all, and Figlu-test, when performed in 3 cases where deficiency might be suspected, was normal

Hypochlorhydria of gastric secretions are well known to be associated with tongue mucosal atrophy as seen by inspection of the oral cavity Studies involving mucosal biopsies have been reported by Wilkinson and Oliver (24), Oatway and Middleton (14) and Witts (25)

In the present study 57 patients suffering from various diseases were examined for the presence of free hydrochloric acid in their fasting gastric secretions by a routine histamine stimulation test Maximal gastric secretion tests however were not performed Among 34 patients with hypochlorhydria 21 had atrophic pattern at tongue biopsy In another group consisting of 23 patients with free hydrochloric acid in their gastric secretions only 8 had tongue atrophy Thus normal tongue histopathology was found in the two groups in 13 and 15 patients respectively There is however no doubt that a certain correlation between gastric hypochlorhydria and an atrophic tongue papillary pattern do exist

Low protein diet may be followed by mucosal atrophy Stein and Spencer (19 observed the beginning of atrophy after one month and fully developed after 4 months Regeneration was complete after vitamin and protein supply This is in accordance with the findings in 6 cancer patients, by Stein and Gold (18), and in a similar case in our study Prolonged tissue hypoxia may possibly give rise to atrophic changes of the tongue mucosa, (6, 16, 23) However, our study does not so far offer a clear answer to this question

Summary and conclusion

The study of 83 tongue biopsies performed in 70 patients including 7 controls is reported Among 63 patients suffering from various diseases within internal medicine 37 presented a pathological tongue mucosa dominated by the same lesion a slightly developed or advanced atrophy of the filiform and fungiform papillae

The macroscopical appearance of the tongue surface was in extremely good agreement with the microscopical picture, the degree of atrophy however was better classified histologically

The atrophic pattern was found in patients with anaemia and/or low serum iron concentration and absence of free hydrochloric acid in gastric secretions Tongue mucosal atrophy was also seen in malignancy in diseases combined with malnutrition including one case of hypovitaminosis B_1 however absent in another case of hypovitaminosis C In pernicious anaemia and in sideropenia the atrophic pattern was changed to that of lively regeneration a few days after initiation of adequate therapy Most of the patients with psychosomatic gastrointestinal disorders diabetes mellitus and chronic bronchitis had a normal tongue mucosa at histological examination Mucosal atrophy of the tongue of

the same type was also observed in single representative cases of amyloidosis, advanced dermatomyositis, Sjogren's syndrome and medicine abuse

It is concluded that papillary atrophy is the predominant reaction of the tongue mucosa in several groups of chronic diseases The atrophy is non specific and qualitatively independent of the underlying cause

Mucosal atrophy of the tongue as an isolated clinical finding may point to the presence of a latent serious disease The efficiency of prolonged therapy for instance in pernicious anaemia and sideropenia may be checked by the histopathological pattern of the tongue mucosa

Acknowledgement

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Studies on Iron Absorption III

By

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Anaemia is almost invariably present in patients with chronic renal insufficiency and raised blood urea (1) The cause (or causes) of this anaemia is uncertain, but all workers agree that the bone marrow function is depressed (4) It has been suggested that this depression is caused by an inadequate production or release of erythropoietin in the kidneys (3) There is further evidence that the erythropoietic tissue can no longer respond to erythropoietin (2) More probably the depression is a result of the toxic influence of azotaemia on the bone marrow (2) A number of investigators have demonstrated a decreased red cell survival in uraemia (4) and an extracorpuscular factor has been found to be responsible for this haemolysis (7)

The anaemia in uraemic conditions is most frequently normochromic and normocytic The bone marrow may appear to be hypoplastic or normal The serum iron values are usually low and the total iron binding capacity is reduced

In order to investigate how iron is absorbed and also whether ascorbic acid stimulates iron absorption in uraemic patients, the following experiments were carried out.

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Methods

A number of patients suffering from renal insufficiency with uraemia but without actual bleeding were examined Based on history and clinical findings all cases were supposed to have a chronic glomerular nephritis They had a low fasting serum iron levels a normo- or slightly hypochromic anaemia with a slightly hypoplastic bone marrow On two successive days the patients received 300 mg ferrous fumarate on the 1st day with the addition of 0 5 g ascorbic acid on the 2nd day Serum iron was recorded fasting and after 2 4 and 6 hours The patients took no food during the experiments (6) For comparison 4 patients with iron deficiency were examined in the same way

Results

Table I shows the results The rise in serum iron on the 1st day is below 20 μg % for 7 of the uraemic patients and 38 μg % for the 8th patient (mean 14 μg %) On the 2nd day the rise is more than 75 μg % in 6 patients 51 μg % in 1 and 20 μg % in the last one (mean 75 μg %) In the patients with iron deficiency anaemia the mean rise in serum iron values is 92 μg % on the 1st day and 198 μg % on the 2nd

TABLE I The effect of ascorbic acid on iron absorption in uraemic patients The values set in italics represent fasting serum iron Where 3 (respectively 4) serum iron values are given, the serum iron has been estimated 3 and 5 (respectively 2, 4 and 6) hours after the fasting values — and after the patients had received their test dose of iron (300 mg ferrous fumarate) on the 1st day, and the test dose+0 5 g ascorbic acid on the 2nd day

Patient no	Diagnosis	Hb (g %)	Blood urea (mg %)	Serum iron values (μg %) 1st day	2nd day
1	Glomerulonephritis chronica	8 1	323	*32* 32, 46	*36* 78 87
2	Glomerulonephritis chronica	8 1	154	*48*, 52, 40 44	*54* 111 131, 131
3	Glomerulonephritis chronica	9 3	171	*67* 76, 74 65	*57*, 77, 124 175
4	Glomerulonephritis chronica	9 4	392	*63* 60, 75	*67*, 74 87
5	Glomerulonephritis chronica	8 3	242	*47*, 85 80	*45*, 117, 120
6	Glomerulonephritis chronica	9 2	151	*78*, 87, 80	*79*, 162, 171
7	Glomerulonephritis chronica	9 0	164	*87*, 85, 103	*55* 125, 130
8	Glomerulonephritis chronica	10 5	205	*78*, 65, 68	*86*, 157 167
9	Anaemia sidero — penica	9 4	Normal	*45*, 50 155 184	*42*, 273 317
10	Anaemia sidero — penica	10 5	Normal	*75*, 82, 101	*59*, 183, 177
11	Anaemia sidero — penica	12 5	Normal	*56*, 63 178	*63*, 344, 323
12	Anaemia sidero — penica	8 3	Normal	*34*, 83, 104	*51*, 110, 185

Discussion

The value of performing an iron absorption test of the type mentioned is discussed in an earlier paper (5) It does not seem reasonable to think that an increase in serum iron has been caused by anything but iron absorption It is shown that the iron absorption varies very little from one day to another (5), but after a series of iron doses the iron absorption may be slightly reduced (6) That is the reason why the sequence of iron, and iron together with ascorbic acid, has not been changed during the experiments All the uraemic patients examined had anaemia, low fasting serum iron and showed a very poor iron absorption Nevertheless, in 7 of the 8 patients, convincingly higher serum iron levels were reached after the addition of ascorbic acid The mean value of this increase was about 60 % of the corresponding rise in the patients with iron deficiency anaemia

Summary

In 8 patients with chronic renal insufficiency, anaemia and uraemia, low fasting serum iron and poor iron absorption were found By adding 0 5 g of ascorbic acid to an orally administered dose of 300 mg ferrous fumarate, the serum iron levels increased with a mean value of 75 μg %

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Acta Medica Scandinavica Vol 178, fasc 5, 1965

From King Gustaf V Research Institute, Stockholm, Sweden

A 6 S Fragment of the Rheumatoid Factor Giving Rise to Hemagglutination

Preliminary Report

By

NANNA SVARTZ

In Acta Medica Scandinavica 177 213 1965 my coworkers and I reported on different breakdown products of the rheumatoid factor (RF)

On fractionating the RF with ammonium sulphate in 1 23 molar concentration a fraction appeared which had retained its ability to induce hemagglutination This fragment, on ultracentrifugation showed a sedimentation coefficient between 12 and 16 S instead of the original 18 7 S Since it was conceivable that the retention of hemagglutinating ability was due to the fact that the breakdown had not proceeded beyond 12—16 S we considered it necessary to try to achieve further dissociation This was not possible with ammonium sulphate and we therefore tried other methods It was found that continued breakdown of the RF could be brought about by the addition of a solution of sodium phosphate and sodium chloride (NaH_2PO_4 0 05 mol l and NaCl 0 077 mol l⁻) pH 4 4 to the 12—16 S fraction obtained by precipitation of rheumatoid arthritis serum or of purified RF with $(NH_4)_2SO_4$ In this way a fragment was formed with a sedimentation coefficient of around 6 S which proved to have retained the hemagglutinating capacity

Fig 1 shows ultracentrifugation of a fraction of RF after treatment with $(NH_4)_2SO_4$ and fig 2 the final fragment of RF after adding phosphate saline solution to the fraction demonstrated in fig 1 A peak is observed with a sedimentation coefficient of 6 S Fig 3 shows the precipitation line of the original RF on immunoelectrophoresis Fig 4 a shows immunoelectrophoresis when the 6 S fragment of RF in fig 2 was reacting against anti human serum Remarkably enough no precipitation line could be demonstrated Using a strong anti γ G serum the result was the same as also with anti RF or anti γ M i e no precipitation line appeared while RA serum gave rise to precipitation lines

Submitted for publication June 5 1965

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with all the anti-sera (figs 4 a to c) Ultracentrifugation as well as the hemagglutination test (Waaler-Rose) showed that a protein was present in the 6 S solution, but in spite of that no precipitation was demonstrable on immunoelectrophoresis These experiences seem to indicate that fractionation of the RF with ammonium sulphate gives rise to a type of protein which is not present in the human blood since no precipitation line could be obtained with antitotal human serum etc New series of investigations are in progress, e g for determination of N-terminal amino acids of the fractions

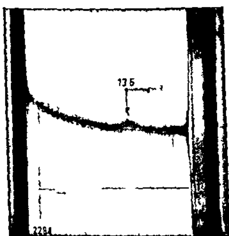
It has thus proved possible to remove the greatest part of the RF macroglobulin without loss of its hemagglutinating capacity The sedimentation coefficient is thereby reduced from around 18 7 S to about 6 S This fragment is for the present called the R fragment of the RF or, abbreviated, RRF

Using cysteine, for example, the breakdown can be carried as far, i e to 6—7 S, but by this method, which implies severing of the disulphide bonds between the peptides, the hemagglutinating capacity disappears completely As already demonstrated in the previous report in Acta med scand this 6—7 S fragment gives rise to a precipitation line in the γ M area, in contrast to the 6 S obtained by (NH_4) SO_4 fractionation

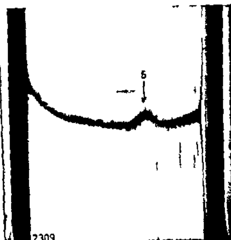
We have also compared our results with the breakdown products obtained by papain digestion of the RF, but have not so far come to compatible results, since the papain solution itself gives rise to a certain hemagglutination

Summary

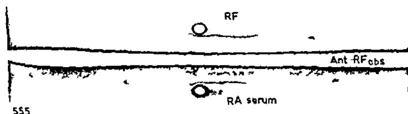
As far as we can discover, fractionation with ammonium sulphate is the best method of dissociating the RF macro globulin without depriving it of its hemagglutinating capacity A further breakdown is obtained through the influence of a solution of prim sodium phosphate and saline on the precipitate from $(NH_4)_2$ SO_4 fractionation of the Rheumatoid factor A hemagglutinating fraction with a sedimentation coefficient of about 6 S is formed It retains the essential property of the Rheumatoid factor, i e it gives rise to hemagglutination but not to any precipitation line on immunoelectrophoresis This 6 S fraction will for the present be called the R fragment of the RF or, abbreviated, RRF



Fg 1 Isclated heumato d factor w h a sed menta on cocff ent of 18 7 S treated w h ammon um sulphate A 13 6 S peak was formed



F g 2 The 13 6 S fragment n f g 1 treated w th prim sod um pl osphate and sal ne A 6 S peak was formed



F g 3 Immunoelectrophores s of the ong nal RF

with all the antisera (figs 4 a to c) Ultracentrifugation as well as the hemagglutination test (Waaler Rose) showed that a protein was present in the 6 S solution, but in spite of that no precipitation was demonstrable on immunoelectrophoresis These experiences seem to indicate that fractionation of the RF with ammonium sulphate gives rise to a type of protein which is not present in the human blood since no precipitation line could be obtained with antitotal human serum etc New series of investigations are in progress, e g for determination of N terminal amino acids of the fractions

It has thus proved possible to remove the greatest part of the RF macroglobulin without loss of its hemagglutinating capacity The sedimentation coefficient is thereby reduced from around 18 7 S to about 6 S This fragment is for the present called the R fragment of the RF or, abbreviated, RRF

Using cysteine, for example, the breakdown can be carried as far, i e to 6—7 S, but by this method, which implies severing of the disulphide bonds between the peptides, the hemagglutinating capacity disappears completely As already demonstrated in the previous report in Acta med scand this 6—7 S fragment gives rise to a precipitation line in the γ M area, in contrast to the 6 S obtained by $(NH_4)_2SO_4$ fractionation

We have also compared our results with the breakdown products obtained by papain digestion of the RF, but have not so far come to compatible results, since the papain solution itself gives rise to a certain hemagglutination

Summary

As far as we can discover, fractionation with ammonium sulphate is the best method of dissociating the RF macroglobulin without depriving it of its hemagglutinating capacity A further breakdown is obtained through the influence of a solution of prim sodium phosphate and saline on the precipitate from $(NH_4)_2 SO_4$ fractionation of the Rheumatoid factor A hemagglutinating fraction with a sedimentation coefficient of about 6 S is formed It retains the essential property of the Rheumatoid factor i e it gives rise to hemagglutination but not to any precipitation line on immunoelectrophoresis This 6 S fraction will for the present be called the R fragment of the RF or abbreviated RRF

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The Triiodothyronine Suppression Test in Hyperthyroid Patients on Antithyroid Medication

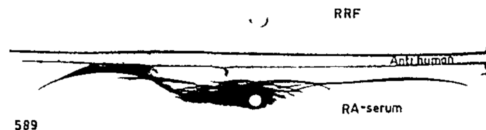
By

THORKILD FRIIS

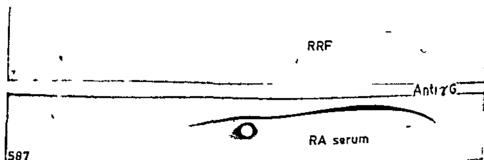
It is generally recognized that l thyroxine and l triiodothyronine nearly always inhibit the thyroid uptake of radioactive iodine in normal, but not in hyperthyroid subjects This circumstance has been utilized clinically the ^{131}I uptake by the gland being determined before and after administration of triiodothyronine or thyroxine If l triiodothyronine is used the period of its administration is one week, with thyroxine considerably longer (5 7 10 11 14 15 17) On the whole there has been little overlapping between normal and hyperthyroid patients and this also applies to two Danish series in which triiodothyronine was used (3 18) Friis (3) found that thyroid function could be suppressed by triiodothyronine in only one out of 34 hyperthyroid patients while among 27 normal subjects it could be suppressed in all but one A similar result has been reported by Ostergard Kristensen et al 18)

As far as treated hyperthyroid patients are concerned the reports are conflicting According to Fellinger (2) euthyroid persons who have had thyroidectomy for hyperthyroidism show normal appearances This has also been reported by Morgans et al (9) while Ostergård Kristensen et al (18) *state that frequently the glandular function in thyroidectomized previously hyperthyroid patients cannot be suppressed In respect* to ^{131}I treated hyperthyroid patients who have been *rendered* euthyroid Hales et al (6) and Fellinger (2) found normal values There are only scanty reports on the result of the tests in hyperthyroid patients during or after *treatment* with antithyroid drugs According to Hales et al (7) these patients thyroid function cannot be suppressed by triiodothyronine as long as they *are toxic* Morgans et al (9) have reported that after completion of treatment 4 patients behaved like normal subjects and Werner (13) got varying results in 9 patients even though clinically they were euthyroid Vander Laan et al (12) state that in 27 out of 32 patients the thyroid function could be suppressed by l thyroxine after one year s treat

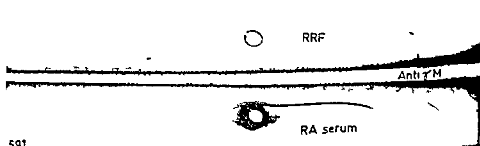
Submitted for publication May 6 1965



a



b



c

Fig. 4 a) Immunoelectrophoresis of the 6 S fragment (RRF) in fig. 2. No precipitation line with anti human serum. b) The 6 S fragment reacting against anti γG. No precipitation. In the lower half the typical γG precipitation line appears. c) The 6 S fragment reacting against anti γM. No precipitation. In the lower half γM precipitation.

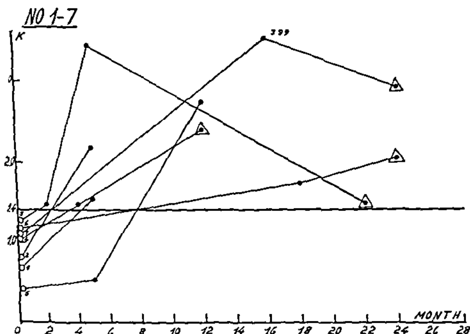


Fig 1 Course of K value in hyperthyroid patients on medication Cases 1—7 (who did not develop recurrences) Abscissa Months Ordinate K value ○ Hyperthyroid before or during treatment. ● Euthyroid during treatment △ Euthyroid after completion of treatment (The figures on the left at the beginning of the curves indicate case numbers)

Results

The material falls into 2 groups as hyperthyroidism recurred in 17 of the 37 patients after medication had been discontinued The severity of hyperthyroidism was originally approximately the same in both groups as assessed on the basis of B M R and clinical signs It may be mentioned that among the patients who had no recurrence, 7 had no palpable goitre 8 had diffuse goitre and 5 had adenomatous goitre Five had mild exophthalmos, 1 severe malignant exophthalmos and 3 had been thyroidectomized for hyperthyroidism Among the 17 patients with recurrences 4 had no goitre 6 diffuse goitre, 7 adenomatous goitre, 1 mild exophthalmos, and 1 had been thyroidectomized All the cases of hyperthyroidism were mild or moderate There was no difference in treatment period between the two groups In the group of patients without recurrences it was 4—53 months m = 12 2 months, and in those with recurrences 4—18 months m = 10 4 months

A Patients without recurrences (Nos 1—20)

Figs 1—4 record the K values in the patients who did not develop recurrences In 16 these values were determined before the medication was started Nos 1—7 are illustrated in fig 1 All

ment In the remaining 5 the hyperthyroidism recurred later, and in these authors' opinion the test is of prognostic value in foretelling a recurrence

There has been a good deal of discussion as to which factor decides whether or not thyroid function can be suppressed by thyroid hormone Various findings appear to indicate that in hyperthyroid patients the thyroid gland is not merely under the influence of the thyrotrophic hormone, but also governed by a special hormone LATS (long acting thyroid stimulator) whose site of formation is unknown and which differs from the thyrotrophic hormone in exerting a more long-lasting action upon mouse thyroids and in not being neutralized by thyrotrophic hormone antibody (1, 8) Allegedly, thyroid hormone cannot suppress the secretion of this hormone, unlike that of thyrotrophin

Since, accordingly, the reports on the triiodothyronine suppression test in hyperthyroid patients on antithyroid medication are conflicting I have studied this problem with a particular view to the prognostic value of the test

Material and method

The material comprises 37 hyperthyroid patients 31 women and 6 men ranging in age from 21 to 84 years. The patients' condition was assessed clinically every other month, and this was supplemented by determination of the B M R, of P B I, and of the uptake of labelled triiodothyronine by the red cells On the basis of the findings, it was decided whether they were eu or hyperthyroid The medication was methyl thiouracil, 50—200 mg daily In 10 cases this drug had to be discontinued because of untoward side effects in the form of leukopenia, exanthema, and arthralgia, and replaced by Carbimazol (Neo-Mercazole), 5—20 mg daily In 4 patients an existing goitre enlarged The medication was carried on for a period of from 4—53 months. After it had been completed the patients were followed for at least one year with a view to possible recurrence (m = 14 1 months) During the period of medication the triiodothyronine suppression test was done at suitable intervals, so that 2—7 tests were done on each patient. A total of 136 tests were performed The antithyroid medication was not altered while the suppression test was being performed The technique was as follows The 4-hour and 24-hour uptakes of ^{131}I by the thyroid were determined as usual (4) Thereafter 1 triiodothyronine (Tertroxin) 20 μg was administered four times daily for 9 days On the 8th day the residual quantity of ^{131}I in the thyroid was determined and a new dosage (10—20 μC) of ^{131}I was given The 4- and 24-hour uptakes were determined after subtraction of the residual quantity and of 87 % of the residual quantity respectively the effective half life of the residual ^{131}I in the gland being estimated as 6 days Thereupon 2 factors, K_1 and K_2 were calculated in which

$$K_1 = \frac{\text{4 hour uptake of } ^{131}\text{I before administration of } T_3}{\text{4-hour uptake of } ^{131}\text{I during administration of } T_3} \text{ and}$$

$$K_2 = \frac{\text{24-hour uptake of } ^{131}\text{I before administration of } T_3}{\text{24-hour uptake of } ^{131}\text{I during administration of } T_3}$$

The mean value $K = \frac{K_1 + K_2}{2}$ was calculated According to Friis (3) normal K values exceed 1 40 and hyperthyroid K values are lower than 1 40

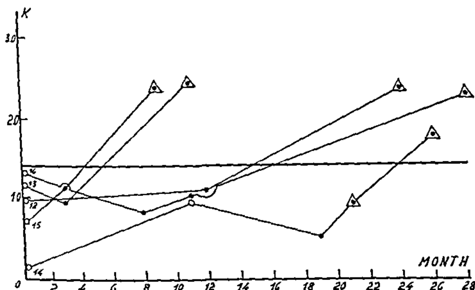


Fig 3 Course of K value in hyperthyroid patients on medication. Cases 12—16 (who did not develop recurrences) Abscissa Months Ordinate K value ○ Hyperthyroid before or during treatment ● Euthyroid during treatment △ Euthyroid after completion of treatment. (The figures on the left at the beginning of the curves indicate case numbers)

for the first time (from 6—33 months) Three were in the abnormal range although they were euthyroid In these patients the K value returned to normal during continued treatment Case 17 had to be thyroidectomized after the 2nd test as the goitre increased very considerably in size Case 20 had malignant exophthalmos which improved on the antithyroid medication This patient was also treated with prednisone In this case too the K value returned to normal All three were tested after completion of the treatment and the K values were still normal

In other words the total results of testing the 20 patients without recurrences were as follows In 15 possibly in 17 the K values returned to normal during antithyroid medication but in 3 not until the medication had been discontinued On the whole however the thyroid function became normal, as estimated by the triiodothyronine suppression test

B Patients with recurrences

By way of comparison, the findings in the 17 patients with recurrences will be described Ten were tested before the medication was started Their K values may be seen from figs 5—8

Fig 5 records cases 21—25 All were thyrotoxic and had abnormal K values before the treatment was started One had 2 recurrences (case 21) 3 (cases 21

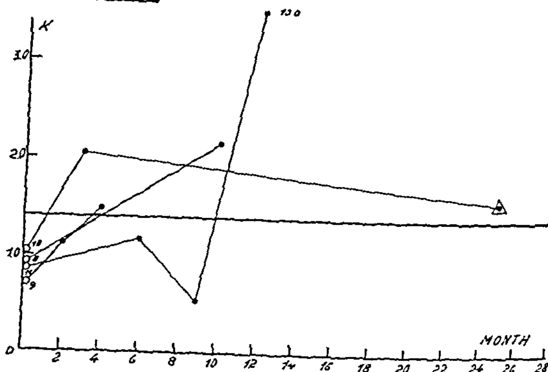


Fig 2 Course of K value in hyperthyroid patients on medication Cases 8—11 (who did not develop recurrences) Abscissa Months. Ordinate K value. ○ Hyperthyroid before or during treatment ● Euthyroid during treatment △ Euthyroid after completion of treatment (The figures on the left at the beginning of the curves indicate case numbers.)

had been tested prior to treatment, all were thyrotoxic and showed abnormal K values During the period of medication, which lasted for from 5—18 months, the K value returned to normal in all cases Four were also tested after completion of the treatment The K values were still normal, i e the thyroid function could be suppressed by triiodothyronine

Fig 2 illustrates cases 8—11 who did not differ fundamentally from the first 7 The treatment period was 4—12 months Fig 3 shows Nos 12—16 These patients too had been tested before the treatment, when they were thyrotoxic, and showed abnormal K values During medication, continued for 5—19 months, the K values failed to return to normal at least in cases 12, 14, and 16, as the treatment was discontinued immediately after the triiodothyronine suppression test had been performed However, the K values returned to normal at a later juncture In cases 13 and 15 the medication was continued for another 2 months after the last suppression test so that K value may have returned to normal during treatment after the test was done When these patients were tested again after completion of the treatment the K values were normal Fig 4, lastly, shows the last 4 patients without recurrences (Nos 17—20) All had received some treatment when tested

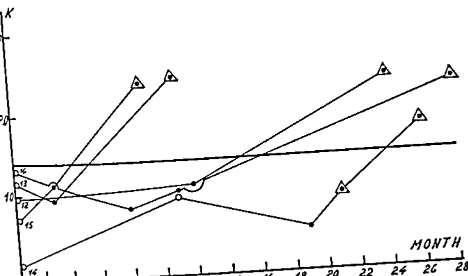


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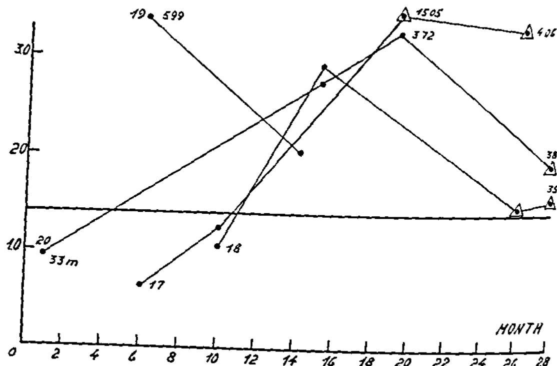


Fig 4 Course of K value in hyperthyroid patients on medication Cases 17—20 (who did not develop recurrences) Abscissa Months Ordinate K value ○ Hyperthyroid before or during treatment ● Euthyroid during treatment △ Euthyroid after completion of treatment (The figures on the left at the beginning of the curves indicate cases numbers The figures at the end of the curves number of months)

22, and 24) had recurrences at the time of the triiodothyronine test The recurrences developed from 3—14 months after the treatment had been discontinued The treatment periods ranged from 5 to 17 months It is worth mentioning that in no case did K rise to the normal range at any time during or after the medication was withdrawn

Fig 6 sets out cases 26—30 All were tested before the medication was started, and all were found to be thyrotoxic, having low K values In two (cases 26 and 27) the K value returned to normal after treatment of the recurrence was discontinued, but it had not been normal at any time prior to the recurrence In cases 28—30, however, the K value was found to be normal before the recurrences appeared At the time of the recurrence, the suppression test was done and again showed abnormal K values Recurrences manifested themselves 4—6 months after the treatment had been discontinued The treatment periods were 4—14 months

Fig 7 illustrates cases 31—34 These patients had been on medication for 1—5 months before the first test In all the K values were abnormal All were euthyroid when tested for the first time Two developed 2 recurrences (cases 31 and 33) In one (case 31) the K value was abnormal throughout,

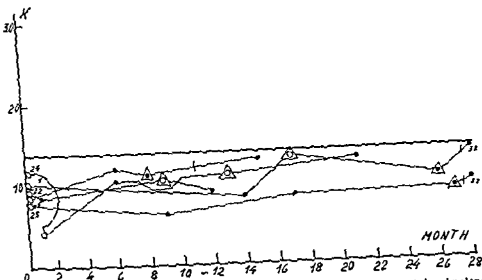


Fig 5 Course of K value in hyperthyroid patients on medication Cases 21—25 (who developed recurrences) Abscissa Months Ordinate K value ○ Hyperthyroid before or during treatment. ● Euthyroid during treatment △ Euthyroid after completion of treatment (△) Hyperthyroid after completion of treatment (Recurrence during suppression test) [Recurrence during which no suppression test was done (The figures on the left at the beginning of the curves indicate case numbers The figures at the end of the curves number of months)

during as well as after treatment In two (cases 32 and 33) the K values returned to normal during the treatment period after the recurrences while during the recurrences they were abnormal In one (case 34) the K value had returned to normal before the recurrence The treatment periods were 5—16 months and the recurrences manifested themselves 2–18 months after the medication had been discontinued Only one patient (case 33) had the triiodothyronine suppression test during the course of a recurrence

Fig 8 lastly sets out cases 35—37 also not tested before the institution of treatment These patients had been on medication for from 6—18 months before the first suppression test which showed in all K values within the normal range, and accordingly the medication was discontinued Thereafter case 35 showed an abnormal K value before the recurrence appeared Cases 36 and 37 also had recurrences after treatment had been discontinued Suppression tests were carried out during the recurrences and showed abnormal K values One (case 37) had ¹³¹I treatment which resulted in normalization of the K value It may be mentioned that in two of the patients a very long time passed between discontinuation of the treatment and the appearance of the recurrences

NO 26-30

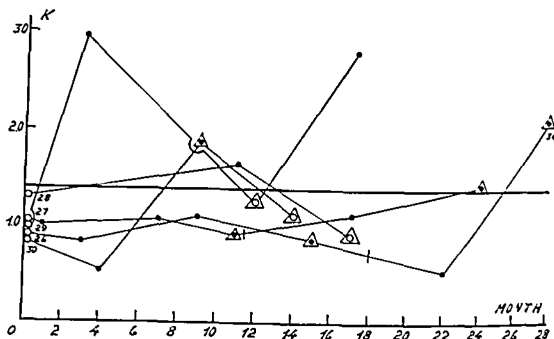


Fig 6 Course of K value in hyperthyroid patients on medication Cases 26—30 (who developed recurrences) Abscissa Months Ordinate K value ○ Hyperthyroid before or during treatment ● Euthyroid during treatment △ Euthyroid after completion of treatment △ Hyperthyroid after completion of treatment (Recurrence during suppression test) | Recurrence during which no suppression test was done (The figures on the left at the beginning of the curves indicate case numbers The figures at the end of the curves number of months)

On the whole, it must be emphasized that among the 17 patients with recurrences 7 (41 %) showed normal K values before the recurrence appeared It may be added, however, that in two the recurrences did not occur until 2—3 years after the medication was discontinued

Conclusion and discussion

Among the 20 patients who did not relapse after discontinuation of the treatment, at least 15 (75 %), and possibly 17 (85 %), showed normalization of the K values during the medication, 3 (15 %) not until after the medication had been withdrawn All 16 patients tested before the institution of the treatment showed abnormal K values At the time all 16 were hyperthyroid This is in keeping with previous findings that the thyroid function cannot be suppressed in hyperthyroid patients (int al 3 15, 16, 18)

Among the 17 patients in whom hyperthyroidism recurred after discontinuation of the treatment, 10 — all thyrotoxic at that time — had tests for the K value before treatment which proved abnormal in all In 3 of these 10 patients the K value returned to normal before the recurrences appeared In 5 the K value remained abnormal

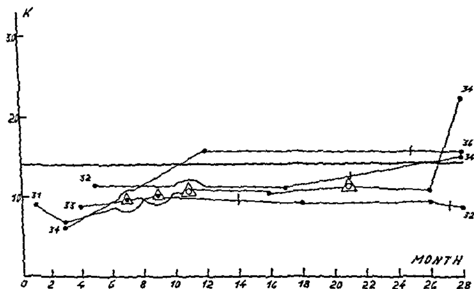


Fig 7 Course of K value in hyperthyroid patients on medication. Cases 31—34 (who developed recurrences) Abscissa Months Ordinate K value ○ Hyperthyroid before or during treatment. ● Euthyroid during treatment △ Euthyroid after completion of treatment. Hyperthyroid after completion of treatment (Recurrence during suppression test) | Recurrence during which no suppression test was done. (The figures on the left at the beginning of the curves indicate case numbers The figures at the end of the curves number of months)

throughout while in 2 it returned to normal after the recurrences had been treated Among the 7 patients whose K values were not tested before the treatment 4 showed normal values before having their recurrences 2 during the treatment of their recurrences while in one the K value remained reduced throughout In other words the K value had returned to normal in 7 out of 17 before the recurrences 41 %)

Within the entire series the K values were normalized during medication in 15 + 7 22 possibly 17 + 7 = 24 (65 % Out of these patients 7 developed recurrences 29 %) In 3 + 10 possibly 5 + 10 the K value failed to return to normal during the medication before recurrence if any Ten patients had recurrences (77 %) Therefore if hyperthyroid patients are on antithyroid medication and have been on this treatment for 6—12 months a normal K value indicates that one quarter to one third of the patients of this series are going to develop recurrences after discontinuation of treatment, while an abnormal K value indicates that two-thirds to three-quarters will develop recurrence In other words, determination of the triiodothyronine suppression test appears to be of some though limited prognostic value in respect to the risk of recurrence when the

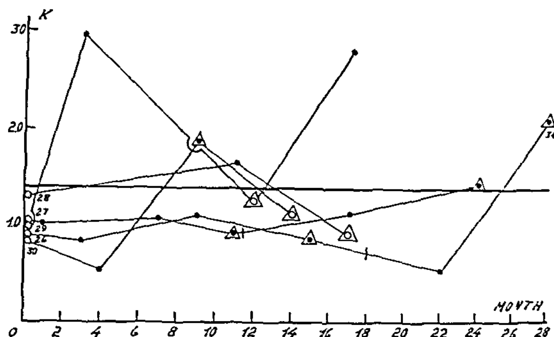


Fig. 6. Course of K value in hyperthyroid patients on medication. Cases 26—30 (who developed recurrences). Abscissa: Months. Ordinate: K value. ○ Hyperthyroid before or during treatment. ● Euthyroid during treatment. △ Euthyroid after completion of treatment. △ Hyperthyroid after completion of treatment. (Recurrence during suppression test.) | Recurrence during which no suppression test was done. *(The figures on the left at the beginning of the curves indicate case numbers.* The figures at the end of the curves number of months.)

On the whole it must be emphasized that among the 17 patients with recurrences 7 (41 %) showed normal K values before the recurrence appeared. It may be added, however, that in two the recurrences did not occur until 2—3 years after the medication was discontinued.

Conclusion and discussion

Among the 20 patients who did not relapse after discontinuation of the treatment at least 15 (75 %) and possibly 17 (85 %) showed normalization of the K values during the medication, 3 (15 %) not until after the medication had been withdrawn. All 16 patients tested before the institution of the treatment showed abnormal K values. At the time all 16 were hyperthyroid. This is in keeping with previous findings that the thyroid function cannot be suppressed in hyperthyroid patients (int. al. 3, 15, 16, 18).

Among the 17 patients in whom hyperthyroidism recurred after discontinuation of the treatment, 10 — all thyrotoxic at that time — had tests for the K value before treatment which proved abnormal in all. In 3 of these 10 patients the K value returned to normal before the recurrences appeared. In 5 the K value remained abnormal

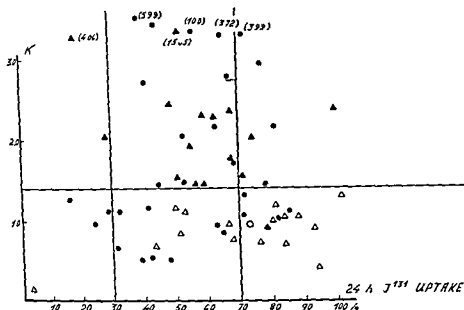


Fig 9 Relationship between K value and 24 hour I^{131} uptake by the thyroid gland in patients who did not develop recurrence ○ Treated hyperthyroid ● Treated euthyroid △ Untreated hyperthyroid ▲ Untreated euthyroid

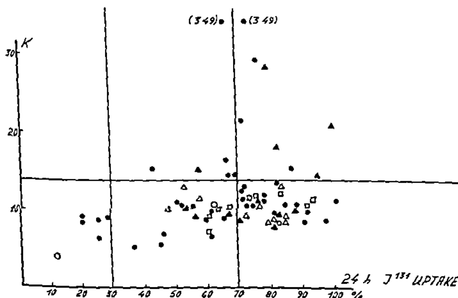


Fig 10 Relationship between K value and 24 hour I^{131} uptake by the thyroid gland in patients who developed recurrence ○ Treated hyperthyroid ● Treated euthyroid △ Hyperthyroid before treatment □ Hyperthyroid after treatment ▲ Untreated euthyroid

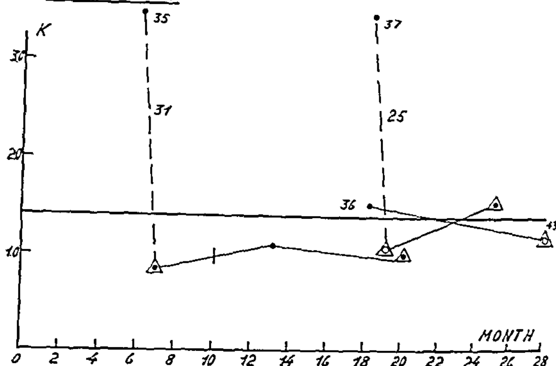


Fig 8 Course of K value in hyperthyroid patients on medication. Cases 35—37 (who developed recurrences) Abscissa Months Ordinate K value ○ Hyperthyroid before or during treatment ● Euthyroid during treatment △ Euthyroid after completion of treatment △ Hyperthyroid after completion of treatment (Recurrence during suppression test) | Recurrence during which no suppression test was done (The figures on the left at the beginning of the curves, indicate case numbers The figures at the end of the curves number of months Figures along broken lines the time interval in months)

treatment is discontinued after 6—12 months medication Our results are not as good as Vander Laan et al 's, who found that all 5 patients whose thyroid function could not be suppressed developed recurrences, which did not appear if the thyroid function could be suppressed

In respect to the ^{131}I uptakes before the administration of triiodothyronine during the suppression tests, the following may be stated If the K values and the 24-hour iodine uptake before administration of triiodothyronine are plotted against each other, there is no relationship among patients who do not develop recurrences (fig 9) The majority of the K values during and after treatment were above the line corresponding to K = 1 40, while before treatment all were below The 24-hour uptakes for patients on medication were as follows reduced in 3 normal in 19, and increased in 9 The corresponding findings for the 4-hour uptakes were 2 13 and 16 Among the patients who relapsed there appeared to be a correlation between the ^{131}I uptake during medication and the K value, an increasing ^{131}I uptake being combined with an increasing K value In other words it seemed that particularly when the uptake was

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increased this could be suppressed by triiodothyronine It may be mentioned that during or after the treatment the K values of most of the euthyroid patients, unlike those of the patients who had no recurrences, were below the K line = 1 40 The 24-hour uptakes were distributed as follows in the patients on medication 6 had reduced values, 16 normal, and 20 increased The corresponding values for the 4-hour uptakes were 0, 22, and 20 Thus, the patients who develop recurrences seem to be more apt than those who do not develop recurrences to have an increased 24 hour ^{131}I uptake during the treatment However, the ^{131}I uptakes alone do not permit of any prognostication in respect to recurrence

The possible presence of goitre and the fact whether or not the patients had been thyroidectomized did not appear to influence the result of the suppression test in the present series As already mentioned, 14 patients had diffuse goitre, 12 nodular goitre, and 4 had been thyroidectomized As far as exophthalmos is concerned, 6 had mild exophthalmos and 1 malignant exophthalmos (case 20) Mild exophthalmos had no influence upon the course of the suppression test In the case with malignant exophthalmos the suppression test became normal, when the exophthalmos improved

Summary

The triiodothyronine suppression test was done at intervals on 37 hyperthyroid patients on antithyroid medication A total of 136 tests were performed After the medication was discontinued, 17 of the patients relapsed The follow up period was a minimum of one year after the medication was discontinued The treatment period ranged from 4 to 56 months, and did not differ in length among patients who developed recurrences and those who did not Among the latter, the triiodothyronine suppression test returned to normal in 17 out of 20 during the treatment (85 %), and in 3 after the treatment had been discontinued Among the patients who developed recurrences, the triiodothyronine suppression test returned to normal in 7 out of 17 (41 %) before the recurrences manifested themselves Thus, in a total of 24 out of 37 (65 %), the triiodothyronine suppression test returned to normal during the treatment In 7 of these cases (29 %) the hyperthyroidism recurred In 13 cases (35 %) the test did not return to normal, and of this group 10 (77 %) recurred It is concluded that the triiodothyronine suppression test had some, though limited, prognostic value, when done after 6—12 months' antithyroid medication, in estimating the likelihood of recurrence after discontinuation of the medication

Acknowledgement

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Urinary Excretion of Porphyrin Precursors in Myocardial Infarction

By

PENTTI KOSKELO and JUHANI HEIKKILA

In addition to the porphyrias there are many diseases and poisonings in which there occurs an increased excretion of porphyrins (Goldberg and Rimington (2)) It has been shown by Koskelo (4) that myocardial infarction belongs to the last mentioned group In a series of 51 cases of acute myocardial infarction there was a significant increa e in the urinary coproporphyrin excretion in 47 patients equivalent to about 92 per cent of the cases

The purpose of the present investigation was to investigate whether in myocardial infarction there is in addition to the increased coproporphyrin excretion any change in the urinary excretion of the porphyrin precursors δ aminolaevulic acid and porphobilinogen

Material and methods

The series comprised 21 patients of myocardial infarction The diagnosis was based on the usual clinical criteria All doubtful cases were excluded from the series The ages ranged from 42 to 76 years the mean age being 54 years There were 16 male and 5 female patients Thirty five apparently healthy ambulatory subjects served as controls

The urine was collected during each 24 hour period stored alkaline and protected from light Determinations of δ aminolaevulic acid (ALA) and porphobilinogen (PBG) were made according to the method of Mauzerall and Granick (5) The results are stated in mg/24 hrs Coproporphyrin determinations were carried out and the calculations were made as described by Koskelo (4) The results of the coproporphyrin analyses are stated in μg/24 hrs The upper limit of normal by this method is 124 μg/24 hrs All the analyses were made immediately after the completion of collection

Submitted for publication Apr l 15 1965

Results

Healthy persons The results for healthy persons are shown in table I The mean 24 hour urinary excretion of ALA in 35 healthy ambulatory subjects was 2 32 mg The values varied in individual cases over a range of 0 50 to 3 72 mg/24 hrs The standard deviation of the mean was 0 77 Values lying between 0 78 to 3 86 mg per 24 hrs were regarded as

An *International Conference on Diabetes in the Tropics* will be held in Bombay from January 20 to 22, 1966

Chairman of the Congress Dr J C Patel

Principal subjects of the Congress

1 Incidence of diabetes, its mortality and morbidity in the tropics

2 Diabetes in the young

3 Pancreatic diabetes

4 Diet with particular reference to high carbohydrate content in diabetes in the tropics

5 Complications of diabetes and their prevention

6 Management of diabetes in the tropics

7 Prevention of diabetes

Organizing Secretary Dr N G Talwalkar, World Congress on Diabetes in the Tropics, The Diabetic Association of India, Maneckji Wadia Bldg, Mahatma Gandhi Road, Bombay I, India

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Two week Course — Cancer Chemotherapy

The Division of Continuing Education, The University of Texas Graduate School of Biomedical Sciences at Houston in collaboration with The University of Texas M D Anderson Hospital and Tumor Institute is presenting a two week course in Cancer Chemotherapy, May 9 to 21, 1966 The chemistry, pharmacology and clinical applications of the antimetabolites, alkaloids, alkylating agents, antibiotic hormones and a miscellaneous group of newer drugs will be reviewed as well as the approaches in current clinical drug assessment and the management of the cancer patient The course will be a comprehensive, in-depth review of the subject

For further information write to Division of Continuing Education, The University of Texas Graduate School of Biomedical Sciences at Houston, 102 Jesse Jones Library Building, Texas Medical Center, Houston Texas 77025

Der 15 deutsche Kongress für ärztliche Fortbildung

wird in der Zeit vom 1 bis 5 Juni 1966 wieder in West Berlin stattfinden

Der Kongress wird am 31 Mai 1966 eröffnet

Auskunft erteilt Kongressgesellschaft für ärztliche Fortbildung e V, 1 Berlin 41, Klingsorstr 21

Acta Medica Scandinavica Vol. 178, fasc 6, 1965

From the Third Department of Medicine University of Helsinki, Helsinki, Finland

Urinary Excretion of Porphyrin Precursors in Myocardial Infarction

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TABLE I Urinary ALA and PBG in healthy persons

Subject No	Age	Sex	ALA (mg/24 hrs)	PBG (mg/24 hrs)
1	37	♀	2 05	1 19
2	54	♂	3 02	0 95
3	65	♂	2 75	1 37
4	49	♂	3 29	1 74
5	34	♀	2 58	2 09
6	32	♂	2 45	2 04
7	33	♂	2 82	2 41
8	23	♀	3 18	1 26
9	74	♀	1 09	1 32
10	52	♂	1 20	1 31
11	43	♂	0 70	1 65
12	65	♂	1 34	3 60
13	67	♂	3 36	2 50
14	73	♀	0 83	2 20
15	63	♀	3 02	1 35
16	40	♀	2 18	3 03
17	22	♀	2 21	1 66
18	29	♀	2 54	2 16
19	28	♀	2 52	1 22
20	59	♀	2 57	1 83
21	60	♂	2 59	1 34
22	23	♂	2 89	1 06
23	47	♀	3 07	2 22
24	39	♂	2 54	1 25
25	59	♂	3 72	1 68
26	48	♂	2 74	1 95
27	39	♂	2 65	2 02
28	23	♀	2 41	2 25
29	45	♀	2 23	2 30
30	48	♂	2 04	1 17
31	51	♂	1 92	0 96
32	57	♂	2 15	1 37
33	53	♀	0 50	0 80
34	58	♂	1 74	1 52
35	46	♂	2 25	1 86

normal, this range being obtained by multiplying the standard deviation on both sides of the mean by two The corresponding values for PBG were mean 1 73 mg/24 hrs, range 0 80 to 3 60 mg/24 hrs, and standard deviation of the mean 0 61 The normal values for PBG were 0 51—2 95 mg/24 hrs

Patients with myocardial infarction The results are shown in table II Elevated

TABLE II Urinary ALA (mg/24 hrs) PBG (mg/24 hrs) and coproporphyrin (UCP μg/24 hrs) in cases of acute myocardial infarction The values above the upper limit of normal are printed in bold type Two dots denote that no analysis was made

Case No	Age	Sex		Days since infarction									
				1	2	3	4	5	6	7	8	9	over 10
1	55	♂	ALA			5 07	3 18	3 57	9 50				
			PBG			1 65	1 44	1 58	2 58				
			UCP			167	133	271	225				
2	60	♀	ALA				2 09	1 19	4 74			2 63	1 91
			PBG				2 42	1 37	1 80			1 47	2 74
			UCP				246	173	99			159	85
3	58	♀	ALA		3 88	1 08	1 70	2 03					
			PBG		1 63	0 96	1 28	2 25					
			UCP		201	160	229	80					
4	57	♀	ALA			4 09		5 06					
			PBG			1 53		2 46					
			UCP			188		238					
5	58	♂	ALA			5 14		4 24	8 90				
			PBG			1 61		1 21	1 53				
			UCP			209		193	480				
6	46	♀	ALA			3 30	2 32					3 95	
			PBG			0 48	1 15					2 14	
			UCP			135	61					84	
7	61	♀	ALA			2 23	5 80						3 30
			PBG			0 83	2 08						2 80
			UCP			46	225						39
8	50	♂	ALA	2 49	2 15	2 90							
			PBG	1 00	1 19	1 15							
			UCP	23	41	227							
9	60	♂	ALA		1 56	2 13		1 89					3 01
			PBG		1 43	1 62		1 85					1 44
			UCP		76	171		140					106
10	52	♂	ALA			3 00		2 27			2 68		4 03
			PBG			1 32		1 11			0 89		2 53
			UCP			196		218			247		161
11	56	♀	ALA		2 62	3 09	3 24						
			PBG		0 00	0 90	0 28						
			UCP		223	143	151						
12	52	♀	ALA			1 05	1 17	4 10					
			PBG			1 74	0 89	1 91					
			UCP			185	163	372					

Cont

Table II Cont

Case No	Age	Sex		Days since infarction 1	2	3	4	5	6	7	8	9	over 10
13	76	♀	ALA			0.53	1.72		1.18	1.00			
			PBG			0.39	1.08		0.70	0.67			
			UCP			36	80		63	60			
14	45	♂	ALA		4.85	2.62				1.99		3.06	1.66
			PBG		2.38	1.18				1.85		2.14	1.63
			UCP		291	216				163		164	88
15	47	♂	ALA		2.59	1.57	2.70				3.59		
			PBG		1.95	1.48	1.34				1.44		
			UCP		221	135	135				132		
16	52	♂	ALA		1.55		3.82	3.34	3.71				2.13
			PBG		1.17		1.24	0.68	1.37				1.45
			UCP		79		201	196	203				123
17	60	♀	ALA		3.56	1.95		1.60	3.24	2.44			
			PBG		1.08	1.02		1.83	1.24	1.19			
			UCP		181	200		129	112	111			
18	54	♀	ALA		1.51	2.27	1.49			2.56			4.75
			PBG		1.40	1.43	1.11			1.04			1.76
			UCP		81	74	71			59			58
19	42	♂	ALA					4.96					
			PBG					1.85					
			UCP					151					
20	83	♀	ALA		1.21		0.35						
			PBG		1.43		1.08						
			UCP		31		71						
21	66	♀	ALA			1.00	0.37	1.69		0.73			
			PBG			0.64	1.27	1.68		1.24			
			UCP			94	133	71		76			

excretion of ALA was seen in 12 patients during some stage of disease PBG excretion was consistently normal in all cases studied Elevated coproporphyrin values were encountered in 18 patients There was no clear correlation between the ALA and coproporphyrin excretion however, the highest ALA excretions were seen in those patients who simultaneously excreted large amounts of coproporphyrin A marked fluctuation from day to day was seen in the ALA excretion without any change in the patients clinical condition In case 10 an elevated excretion of ALA was not seen until on the 11th day in hospital A recurring myocardial infarction was established in this case on the 9th day

in hospital It is evident that an increased excretion is less consistently found for ALA than for coproporphyrin Coproporphyrin excretion was clearly elevated on 33 occasions when the ALA excretion was normal, the opposite being seen on three occasions only The five female patients studied showed, in spite of a clinically sure infarction a normal ALA excretion with the exception of case No 18 in whom an elevated excretion was seen on one occasion when the acute stage of the disease already was over

Discussion

The various forms of porphyria and lead intoxication are the only diseases in which urinary ALA is known to be constantly raised (Hæger Aronsen (3)) In human alcoholic subjects there is usually a rise in coproporphyrin excretion and occasionally also in ALA and PBG (Goldberg and Rimington (2) Orten et al (6)) Pathological excretion of ALA in urine in lead poisoning has been attributed to either increased synthesis of this substance or its decreased utilization for porphyrin synthesis The latter is a consequence of the inhibitory effect of lead on ALA dehydrase an enzyme that catalyses the formation of PBG from ALA (3) Apart from the porphyrin synthesis ALA can be metabolized via the succinate glycine cycle (Shemin (7)) Inhibition of enzymes in this metabolic pathway too may be responsible for accumulation and increased excretion of ALA

It is possible that one of the above mechanisms plays a role in myocardial infarction and may explain the fact that many patients excrete increased quantities of ALA in urine The exact mechanism remains, however, obscure We have not been able to explain the variations in the excretion of ALA from one case to another, nor the fluctuations from day to day in the same patients on the basis of the patients clinical condition

Acute reduction in the daily food intake is known to be associated with a marked rise in the excretion of both ALA and PBG in patients suffering from acute intermittent porphyria (Welland et al (8)) Whether reduction of food intake causes elevated excretion of ALA in persons without porphyria is not known It seems however unlikely that the light diet given in hospitals to infarction patients would be able to alter the formation or excretion of ALA

There is a sex difference in the excretion of urinary coproporphyrin, the mean values for men being greater than those for women (Zieve et al (9)) Hæger-Aronsen (3) found no significant sex difference in the urinary excretion of ALA All but one of the five post menopausal female patients in our series had normal and in some instances even subnormal quantities of ALA One had on one occasion an elevated and otherwise a normal excretion It seems possible that the porphyrin metabolism in female patients responds in a different way to myocardial infarction

The increased urinary excretion of coproporphyrin in myocardial infarction is probably the consequence of disturbance in the decarboxylation and oxidation of coproporphyrinogen III to pro-

Table II Cont

Case No	Age	Sex		Days since infarction 1	2	3	4	5	6	7	8	9	over 10
13	76	♀	ALA			0 53	1 72		1 18	1 00			
			PBG			0 39	1 08		0 70	0 67			
			UCP			36	80		63	60			
14	45	♂	ALA		4 85	2 62				1 99		3 06	1 66
			PBG		2 38	1 18				1 85		2 14	1 63
			UCP		291	216				163		164	88
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			PBG		1 17		1 24	0 68	1 37				1 45
			UCP		79		201	196	203				123
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			PBG		1 46	1 43	1 11			1 04			1 76
			UCP		81	74	71			59			58
19	42	♂	ALA					4 96					
			PBG					1 85					
			UCP					151					
20	83	♀	ALA		1 21		0 35						
			PBG		1 43		1 08						
			UCP		31		71						
21	66	♀	ALA			1 00	0 37	1 68		0 73			
			PBG			0 64	1 27	1 68		1 24			
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toporphyrinogen IX (III) There is accumulation of coproporphyrinogen and subsequent excretion of it and of coproporphyrin in urine One or both of the enzymes catalyzing the reaction may be involved, or there may be a relatively lack of acceptor for the hydrogen atoms which are oxidatively removed from coproporphyrinogen The amount and duration of coproporphyrinuria are about the same as in pulmonary embolism (Eskola et al (1)) It is unlikely that the origin of the disturbance in porphyrin metabolism in myocardial infarction is a substance released by the heart muscle

Summary

Urinary excretion of δ aminolaevulic acid (ALA), porphobilinogen (PBG) and coproporphyrin (UCP) were studied in 21 patients suffering from myocardial infarction Excretion of ALA was increased in 12 patients during some stage of the disease PBG excretion was normal in every case The UCP excretion was increased in 18 patients There was no clear correlation between the ALA and UCP excretions

The possible mechanisms which cause this phenomenon are discussed

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Myelomatosis

By

EINAR WOLFF SØRENSEN

The intention of this paper is to report some unusual observations regarding the sedimentation rate (ESR), serum proteins and platelet function in a case of myelomatosis

Extract from the case history

The patient is a man 72 years old Proteinuria and greatly increased ESR have been recorded in the last 8—10 years

1958 The urine contained 0 1—0 3 g % protein No Bence Jones protein Electrophoresis showed peaks of beta and gamma globulin in the urine and a high peak of gamma globulin (5 g %) in the serum

1962–64 The patient repeatedly had periods of troublesome haemorrhages from his gums

1964 Admitted to hospital because of continuous haemorrhages from the gums Retinal haemorrhages were also observed Hb 8 g ESR *160 mm/hour No patholog*ical findings in the peripheral blood The platelet count varied considerably according to the method used Counts on whole blood showed 2—300 000 platelets/mm³ Sia s test was positive and Bence Jones protein was found in the urine Primary bleeding time 30 minutes secondary bleeding time 7 1/2 minutes Clot retraction (Macfarlane) 20 % TP 72 Quick time and Cephalin time revealed normal values The plasma fibrinogen content was normal and there was no evidence of increased fibrinolysis The serum gamma globulin was *10 6 g % The* sternal marrow contained a large number of myeloma cells and X ray examination of the skull showed areas of destruction typical of myelomatosis

The patient thus fulfilled the criteria for diagnosing myelomatosis Of special interest were the finding of poor clot retraction, a normal number of platelets and a normal Quick time and Cephalin time together with a primary bleeding time of 30 min For these reasons the bleeding tendency was thought to be caused by ineffective platelet function

ESR On repeated estimations the ESR was found to be approximately 160 mm/hour when performed at room temperature and at + 37 C When estimated at + 4 C the ESR *was 80 mm/hour Fig 1 demonstrates* the ESR performed in the ordinary manner (0 4 ml citrate to 1 6 ml blood) (tube A) It is clearly seen how the erythrocytes are agglutinated and attached to the glass surface Tube B shows the ESR after addition of 1 10 volume of a low molecular dextran (Rheomacrodex Pharmacia) No agglutina*tion or attachment* of the red cells can be seen

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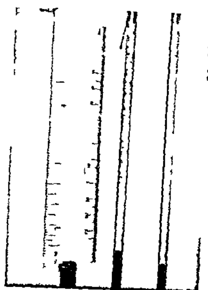


Fig 1 The left tube (tube A) shows the ESR performed in the normal manner (0 4 ml citrate to 1 6 ml blood) The right tube (tube B) shows the ESR after addition of 1/10 volume of Rheomacrodex.

Serum proteins The electrophoretic pattern of the serum proteins showed a contrnt of 10 6 g % gamma globulin (total peotein was 13 3 g %) By ultracentrifugation 10 54 g % gamma globulin was found but no macroglobulin (table I) The findings on ultracentrifugation of a normal serum and the same serum incubated with washed platelets from the patient are shown in the same table Ultracentrifugation of the patient s serum 3 months later showed 10 8 rel % gamma globulin (S = 22 8) After being placed in a refrigerator at − 4 C for some days, the serum from the patient was found to be divided into 2 layers, an upper yellow-coloured and a lower colourless layer Between these layers was a white disk (fig 2) which on ultracentrifugation was found to consist of 70 5 % macroglobulin (S = 12 6) These two last mentioned estimations were kindly performed at Carlsberg Bryggeriet, Copenhagen According to the laboratory the apparent difference in the types of macromolecules is explained by the special treatment of this serum

Throm bocytes Counting of platelets in blood containing pathological proteins with increased viscosity may sometimes be difficult Table II shows the results of platelet counts when blood specimens were allowed to sediment at different temperatures. At various time intervals platelets were counted from different layers in the blood column The platelets sediment more slowly at + 4°C than at + 20 C, but after 1 hour almost all the platelets are found at the bottom of the tubes The influence of different anticoagulants and Rheomacrodex

Table I The results of electrophoresis and ultracentrifugation of the serum proteins The amounts of protein are expressed in gram 100 ml blood except when % is written

	Total protein	Globulin	S-value	Macro globulin	S value
Patient s serum March 1964	13 6	10 54	6 4	0	
Normal serum March 1964	5 7	0 5[illegible]	6 1	0 21	14 8
Patient s platelets incubated with normal serum (0 2/2 ml)	6 5	1 42	6 2	0 23	15 2
Patient s serum July 1964	10 9	9	6 9	10 8 %	22 8
White disk (see text)				70 5 %	12 6



Fig 2 Blood after standing in a refrigerator

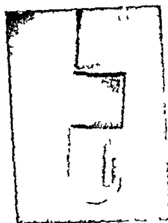


Fig 3 Serum after standing in a refrigerator

on platelet counts in plasma is simply demonstrated in table III

The viscosity of the blood from the patient was measured by means of a drop-counter This method is rather rough but the difference between normal blood and patient s blood is pronounced Even after incubating the patient s blood at + 37 C for 12 hours a difference is still present (table IV)

Table V demonstrates that the clumping time of platelets from the patient is much longer than normal and cannot be normalized by increasing amount of ADP It also shows that the patient s serum lengthens the clumping time of normal platelets.

The ESR results obtained when Rheomacrodex was added to the blood led to the view that administration of Rheo-

TABLE II Number of platelets (in thousands) from different layers in the blood column after sedimentation at room temperature and at +4 C

Sedimentation time	½ hour		1 hour		1½ hour	
Temperature	+20	+4	+20	+4	+20	+4
Layer A (top)	10	7	8	5	14	5
Layer B —	6	170	8	7	14	7
Layer C	13	310	11	9	14	6
Layer D — bottom)	340	400	248	3 8	372	312

TABLE III A comparison of platelet counts in citrated plasma and in EDTA plasma without and after addition of Rheomacrodex The counts are performed on each of three successive days

		1st day	2nd day	3rd day
Citrated plasma		2 600	2 200	2 200
EDTA plasma		60 000	94 000	152 000
EDTA plasma	0 5 ml Rheomacrodex	106 000	120 000	140 000
Citrated plasma	0 5 ml Rheomacrodex	16 400	16 000	11 000

TABLE IV The viscosity of the blood measured by a dropcounter

Normal blood at room temperature	75 drops in 60 seconds
Patient's blood at room temperature	20 drops in 60 seconds
Patient's blood after standing at +37 C for 12 hours	56 drops in 60 seconds

TABLE V A comparison of the clumping time of platelets from a patient with myelomatosis from a normal individual

Experiment no	PRP 0 4 ml		PPP 0 4 ml	ADP		Platelet clumping time in seconds
				10 µg/ml	1 µg/ml	
1	Patient			+0 1 ml		50
2	Normal			+0 1 ml		10
3	Normal	+	Normal	+0 1 ml		10
4	Normal	+	Normal		+0 1 ml	30
5	Patient	+	Patient	+0 1 ml		60
6	Patient	+	Patient		+0 1 ml	no clumping
7	Normal	+	Patient	+0 1 ml		25
8	Normal	+	Patient		+0 1 ml	180
9	Patient	+	Normal	+0 1 ml		60
10	Patient	+	Normal		+0 1 ml	no clumping
11	Patient + Normal			+0 1 ml		10—15
12	Patient + Normal				+0 1 ml	80

PRP platelet rich plasma
PPP platelet poor plasma
In experiments 3—12 the two plasmas had been incubated for 30 min at +37 C

macrodex to the patient might reduce a possible increased tendency to aggregation and stickiness of all blood elements During two episodes when the patient was suffering from continuous haemorrhages from the gums and intestinal tract he was given 500 ml Rheomacrodex daily for 5 days Following this treatment, the haemorrhages stopped the primary bleeding time was reduced to 4 1/2 min, and the clot retraction increased to 38 % The FSR was still 160 mm hour but aggregation or attachment of red cells to the glass could not be seen

Discussion

The observations seem to give some information about the relationship be tween the presence of macroglubin and platelet function In this case the bleed ing tendency appears to be dependent on an inhibition of normal platelet function This inhibition seems to affect the clumping of platelets both from the patient and from a normal person

It is well known that the suspension stability of the blood is highly dependent on the relationship between high and low molecular colloids in plasma (4). An increase in the relative amount of globulins and fibrinogen in plasma in relation to albumin causes a decrease in the stability of the suspension and an increased tendency to blood corpuscle aggregation. It has also been suggested that the presence of abnormal plasma proteins especially macroglobulins may interfere with the platelet function possibly by coating their surfaces and thus preventing contact activation (2, 3). The bleeding tendency associated with macroglobulinaemia of Waldenström is described by Jurgens (2) and Pachter (3) found that the platelets in this condition had a deficient thromboplastic function. It is also shown by Gelin (1) and others that Rheomacrodex, given to a patient with intravascular aggregation will increase the suspension stability of the blood and that this may be accompanied by disaggregation.

It seems reasonable to suppose that a coating of the platelets by macroglobulin has caused the platelet dysfunction in the described case. The administration of Rheomacrodex may have disintegrated these linkages between the platelets and the macroglobulin molecules. This had made the platelets capable of clumping and sticking to the capillary wall in the ordinary manner resulting in a normal haemostasis. Unfortunately the clumping time of the platelets was not tested with addition of Rheomacrodex.

Summary

A brief description is given of a patient suffering from myelomatosis with macroglobulinaemia and bleeding tendency. The haematological findings were normal platelet count, poor clot retraction, bleeding time 30 min., and a normal clotting time, Quick time and Cephalin-time. An observed tendency for erythrocytes to aggregate and stick to a glass surface was eliminated by the addition of 1/10 volume of Rheomacrodex. After treatment with Rheomacrodex infusions, the patient's haemorrhages stopped and the bleeding time became normal. The platelet clumping time was found to be abnormally prolonged. The patient's serum also lengthened the clumping time of normal platelets. The defective platelet function may be caused by the coating of platelets by macroglobulin. This coating seemed to be counteracted by Rheomacrodex resulting in a normal platelet function.

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Efficiency of Haemodialysis

By

F Rényi Vamos S Csata and M Toth

The efficiency of haemodialysis is usually expressed by the difference between the initial and the terminal value of the non protein nitrogen (NPN) Under conditions of undisturbed extracorporeal dialysis the efficiency of the artificial kidney in the possession of this department (type Necker, cellophane surface 2 6 m²) amounts to 70—75 per cent so that for example if the value of NPN is 200 *mg %* *before the intervention* it drops to 50—80 mg % after a dialysis of 4 hours The efficiency of smaller machines is between 50 and 65 per cent in 6 to 8 hours

The decrease in the NPN level of the serum is not uniform during dialysis no matter what type of machine is used It is agreed by all artificial kidney units that efficiency is highest in the first half of the intervention (with our machine in the first two hours and particularly during the first hour) and that the further diminution of the NPN value takes place at a much slower rate to become sometimes quite negligible (table I fig 1) This experience has raised the problem whether it is advisable to continue dialysis over many hours or whether a short treatment should be *preferred since* the diminution of the blood value of NPN during the last hour or hours is out of proportion to the stress the intervention means for the patient

Methods

In order to settle the problem we performed 16 dialyses in which the concentration of NPN was hourly measured both in the blood and in the bath fluid Eight of the 16 treatments lasted 5 hours

It can be seen from table I and the upper curve of fig 1 that the NPN level of the blood decreased in the usual manner the drop was most pronounced in the first hour less rapid in the further course of dialysis and not more than 10 mg % during the last hour

The amount of NPN in the bath fluid changed considerably less unevenly The *elimination of NPN from the blood occurred* at practically the same rate in the first and the second hour the rate of elimination became slower after the second hour but remained constant in the remaining part of the dialysis (table I middle curve fig 1) With a view to making the hourly comparison between the decrease in the NPN level of

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TABLE I. The hourly decrease of the serum NPN level, the hourly NPN concentration of the bath fluid (mean values) and the quotient of the values

Periods (hours)	Decrease of the serum NPN level (mg %)	NPN content of the bath fluid (g/hour)	Decrease of NPN level / NPN content of the bath fluid
1	47 (15–94)	20.5 (7–32)	2.29 (1.1–6.1)
2	38 (16–66)	20.1 (9–30)	1.89 (1.3–4.1)
3	19 (5–33)	16.3 (11–25)	1.16 (0.55–2.40)
4	18 (6–36)	16.0 (6–23)	1.12 (0.50–2.40)
5	10 (4–16)	16.0 (8–20)	0.62 (0.20–1.40)

the blood and the NPN concentration of the bath fluid more expressive, an arbitrary quotient was set up in which the value of the hourly decrease in the NPN level of the blood (i.e. the difference between the level at the beginning and that at the end of the hour) was the numerator (without mg %), and the amount of NPN measured during that hour in the bath fluid (without g) was the denominator. As can be seen from the lower curve of fig. 1 and table I, the value of the quotient descended gradually, except from the 3rd to the 4th hour.

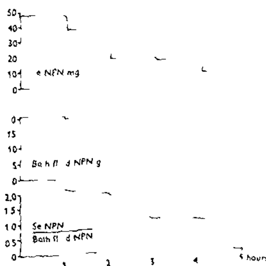


Fig. 1. The hourly decrease of the serum NPN level, the hourly NPN content of the bath fluid (mean values) and the qoutient of the values

Discussion

Concentration of NPN in the blood does not diminish evenly during haemodialysis, if one of our artificial kidneys of large cellophane surface is used; the rate of diminution is highest in the first hour, less pronounced in the following hours, and hardly perceptible or almost nil in the fifth hour.

The hourly harvest of NPN in the bath fluid is, on the other hand, fairly uniform, although the amount of NPN in the fluid is higher than that obtained during the later course of dialysis, the difference between the first and the fourth or fifth hour is insignificant.

A comparison of the decrease in the value of the serum NPN and the amount of NPN in the bath fluid (i.e. the quotient of the two values) makes it evident that while the rate at which the NPN is eliminated from the blood is more or less uniform (or just slightly slower after the second hour), the rate of decrease in the NPN level of the serum becomes slower from hour to hour. This phenomenon was particularly striking in the fifth hour when the decrease

of NPN in the blood was negligible whereas the rate at which NPN was passing from the blood to the bath fluid was not slower than before

Conclusions

1 When testing the efficiency of haemodialysis (and that of artificial kidneys) it is better to measure the amount of NPN in the bath fluid and not the blood values
2 The duration of dialysis should not be curtailed since it is useful to continue the treatment even at a time when the blood value of NPN has practically ceased to decrease
3 Instead of shortening it the duration of the dialysis should be prolonged provided the condition of the patient permits it Led by such considerations we have lengthened the duration of the treatment from 4 to 5 hours in our department because — as has already been noted — although the level of NPN in the serum shows hardly any decrease in the 5th hour the bath fluid of the last hour still contains quite a considerable amount of eliminated NPN (fig 2)

A lengthening of the duration of dialysis has among others the advantage that a repetition of the operation may become superfluous or at least, the number of subsequent dialyses may be lessened

Let us now see why the rate at which the blood level of NPN decreases becomes slower and slower and even drops to zero whereas the rate at which NPN is eliminated from the

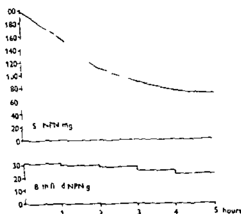


Fig 2 M J 18 year-old Traumatic anuria

blood stream remains — after a slight drop after the second hour — fairly constant throughout the dialysis

Changes in the concentration of NPN in the blood during dialysis depend among others, on two factors 1 the passage of NPN *from the blood stream* to the bath fluid 2 the passage of NPN from the tissues to the blood stream It seems safe to assume that during dialysis outflow of NPN from the blood stream occurs at a fairly uniform rate whereas inflow of NPN from the extravascular compartment is variable The amount of NPN gaining access to the intravascular compartment during the first one or two hours is less than that passing into the bath fluid the consequence is a decrease in the serum level of NPN Inflow from the extravascular space becomes more pronounced in the later course of dialysis so that its rate may reach that of the outflow the consequence is a reduced rate of decrease or even a stabilization of the serum level of NPN

The question arises here as to why it is that a smaller amount of NPN-containing substances gains access to the blood stream at the beginning and a larger towards the termination of dialysis Inducing acute experimental anuria in dogs, we found that, after an ureteral ligation of 3 days, the concentration of NPN in the striated and the smooth muscles varied from 330 to 370 mg % against a mean value of 217 mg % in the serum, while the value in the liver averaged 576 mg % (1) We agree with other authors (2, 3) in that urea and other nitrogenous substances are bound intracellularly, and we think the phenomenon observed in the course of dialysis, as outlined in the foregoing, is mainly due to the uneven distribution of NPN over the intracellular and the extracellular spaces Furhter investigations in this respect are now in progress

Summary

1 When testing the efficiency of haemodialysis (and that of artificial kidneys) it is better to measure the amount of NPN in the bath fluid and not the blood values

2 Concentration of NPN in the blood does not diminish evenly during haemodialysis The rate of diminution is highest in the first hour, less pronounced in the following hours The hourly harvest of NPN in the bath fluid is, on the other hand, fairly uniform

3 It is concluded that the phenomenon observed in the course of dialysis, is mainly due to the uneven distribution of NPN over the intracellular and the extracellular spaces

4 The duration of the dialysis should be prolonged provided the condition of the patient permits it

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Influence of Electrical Supramedullary Stimulation on the Plasma Level of Free Fatty Acids, Blood Pressure and Heart Rate in the Dog

By

LARS ORO, LARS R WALLENBERG and PER BOLME

Several investigations suggest that the sympathetic nervous system plays a role in the mobilization of free fatty acids (FFA) from adipose tissue into blood plasma (cf 22 26) The adrenergic neurohormones, noradrenaline and adrenaline, stimulate the release of fatty acids from adispose tissue *in vitro* as well as *in vivo* (13, 19 20 22) During many conditions with increased sympathetic activity there is also a rise of the FFA level in blood plasma e g during prolonged excercise (2 15 25) during tilting 23 37) and during mental stress (3 5

The effect of carotid occlusion and central vagal stimulation on the plasma level of free fatty acids and the blood pressure in the dog was recently studied by Froberg and Oro (16) The results indicated that the mobilization of FFA to blood plasma was not affected during activation of medullary pressor reflexes

The following investigation was performed to study the possible role of supramedullary regions in the mobilization of FFA to blood plasma The arterial levels of FFA and glycerol in blood plasma and blood glucose were followed during electrical stimulation in the diencephalon and the mesencephalon of anaesthetized dogs Blood pressure heart rate and muscle blood flow were also registered

Methods

General preparations

The experiments were performed on mongrel dogs of both sexes weighing from 9 to 16 kg (mean 13 kg) The dogs were fasted for about 20 hours and anaesthetized with pentobarbitone sodium (Nembutal® Abbott) 25—30 mg/kg b w Supplementary pentobarbitone was given in small doses 3—5 mg/kg b w as soon as the dogs shivered A cannula was inserted into the trachea. The body temperature was kept normal with a

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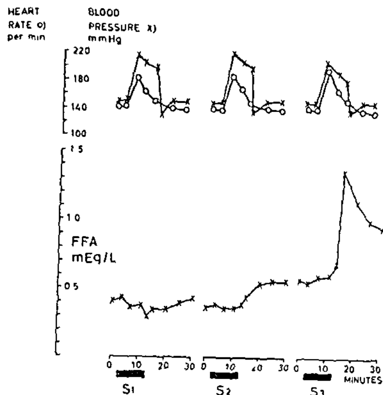


Fig 1 Arterial level of free fatty acids (FFA), heart rate and mean blood pressure during electrical stimulation (S) in a screening experiment (see text) Three different points (1, 2, 3) in the diencephalon and mesencephalon were stimulated during 10 minutes with 6 V The electrode tip was moved 2 mm backwards between each stimulation The last stimulation (S_3) was in the *mesencephalic area*

heating lamp The blood pressure was measured in the right carotid artery by means of a polyethylene cannula connected to a Statham pressure transducer (P 23 AC) The pulse rate was recorded from the blood pressure registration by means of an ordinate writer (18) and the parameters were recorded on a Grass polygraph (Model 5 C)

Stimulation electrodes were introduced into the brain through small holes drilled in the parietal bones, and oriented stereotaxically by means of the Horsley Clarke technique in coordinates taken from an atlas of the dog s brain (30) The electrodes were unipolar, 0 5 mm gauge stainless steel wires, insulated up to 1 mm from the tip The stimuli, delivered from a Grass stimulator (Model S 4 D) had a frequency of 80 imp /sec , a duration of 2 msec and a voltage varying from 1 to 14 V

Blood samples for analysis of FFA glycerol and blood glucose were drawn into heparinized syringes from a cannula in one of the femoral arteries No heparin was injected into the animals before or during the metabolic studies The total blood volume withdrawn from the animals did not exceed 150 ml

At the end of the experiments, the stimulated areas were electrocoagulated via the stimulating electrode The brains were perfused *in situ* through the carotid arteries with 20 per cent formalin, removed and put into 10 per cent formalin Later, the brains were sectioned at a thickness of 0 5—1 mm The localization of the electrode tip was determined by visual examination and the sections were photographed

Screening experiments

Stimulations in diencephalic and mesencephalic regions were performed in a series of screening experiments At the outset chloralose 100 mg/kg b w was used as anaesthetic In these experiments, the level of FFA varied markedly between the different dogs The anaesthetic was therefore changed to Nembutal[R] and only these experiments are included in the results In each dog 3—12 small areas 1—5 mm from the midline at varying depths were stimulated for 5—10 minutes with the same voltage The voltage

ranged in different dogs between 4 and 8 V. In most points the stimulations with this technique did not significantly affect the FFA level in plasma, even when marked changes in heart rate and blood pressure were observed (e.g. S_1 in fig. 1). However the FFA level appeared to increase during stimulation in two circumscribed areas: one in the diencephalon and one in the mesencephalon (fig. 1).

In the preliminary experiments a rise of the FFA level was never observed without a simultaneous increase in heart rate and/or blood pressure. When the two areas were stimulated in the following series of experiments several short (15—30 sec.) stimulations were made in the area at different levels to find the point which most effectively influenced heart rate and blood pressure. If no or only minor cardiovascular changes were observed even with high voltages during test stimulation, the electrode was moved 1—2 mm laterally and/or medially. The test procedure was repeated until a point was found in which the stimulation significantly increased heart rate and or blood pressure. Stimulation of the different points found with this technique in the diencephalon and in the mesencephalon will in the following be referred to as stimulation in the *diencephalic* and *mesencephalic area* respectively.

Stimulation in the diencephalic and mesencephalic areas

In a series of experiments the *diencephalic* and/or *mesencephalic area* were stimulated during 15-minute periods. The levels of FFA and glycerol in blood plasma and blood glucose were followed. In dogs in which both areas were stimulated at least 60 minutes elapsed between the two stimulations.

Stimulation with varying intensity

In a series of experiments the *diencephalic* or the *mesencephalic area* was repeatedly stimulated for 15 minutes with 2 to 4 different voltages. The lowest voltage was chosen to give no or minimal changes in heart rate and blood pressure. The highest voltage always affected blood pressure and/or heart rate. The levels of FFA and glycerol in plasma and blood glucose were followed. At least 45 minutes passed between the stimulations.

Adrenalectomy

Adrenalectomy was performed in a series of experiments. At the beginning of the experiment 25 mg cortisone acetate (Cortone®, Merck Sharp & Dohme) was injected i.m. The *diencephalic area* was stimulated during 15 minutes and the arterial level of FFA was followed. The adrenal glands were removed via incisions in the flanks and 50 mg of hydrocortisone (Solu Cortef® Upjohn) was given i.v. About 60 minutes later the stimulation was repeated. In order to minimize the blood loss in the operated animals no blood was taken for analysis of glycerol.

Sympathetic ganglionic blockade

The *diencephalic* or the *mesencephalic area* was stimulated for two periods of 15 minutes. The levels of FFA and glycerol in blood plasma and of blood glucose were followed. Prior to the second stimulation period a sympathetic ganglionic blocking agent (Agent I®, (Recip), hexamethylene hydroxyethyl dimethylammonium chloride) was given i.v. until short stimulations no longer elicited any circulatory effects. The total dose of Agent I® varied between 25 and 50 mg/kg b.w.

Muscle blood flow

In some experiments the muscle blood flow was determined according to Lindgren (31). At the end of the metabolic study the dog was heparinized with 10-15 mg/kg b.w. intravenously. One of the femoral arteries was cannulated. The blood was directed through a silicone filled drop-chamber and returned via a small artery to the muscles of the thigh. The blood pressure in the perfused muscle was recorded by a Statham pressure transducer (P 23 AC). A screw-clamp was placed around the femoral artery proximal to the drop-chamber. By tightening the clamp the blood pressure in the perfused

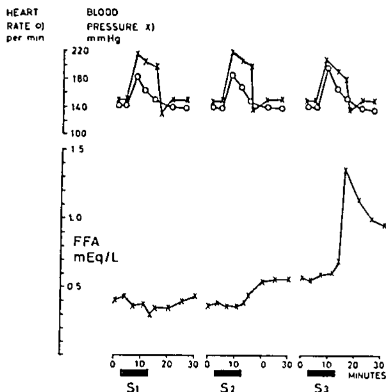


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TABLE II Changes in the levels of FFA and glycerol in arterial blood plasma and blood glucose during and after 15-*minute stimulations in the diencephalic and the mesencephalic areas* The figures are calculated on the individual changes from the mean concentration before the stimulations and represent the mean values and the standard errors of the mean

Minutes after the start of the stimulation			5	10	15	17	22	27
Diencephalic area n = 9	FFA mEq/L	Mean	*0.07	*0.13	*0.18	*0.23	*0.18	*0.13
		SEM	±0.01	±0.04	±0.07	±0.06	±0.04	±0.03
	Glycerol mMol/L	Mean	*0.025	*0.054	*0.067	0.066	*0.051	*0.030
		SEM	±0.008	±0.010	±0.012	±0.010	±0.008	±0.007
	Blood glucose mg/100 ml	Mean	4	8	10	11	12	*11
		SEM	±1	±5	±6	±6	±6	±4
Mesencephalic area n = 8	FFA mEq/L	Mean	*0.10	*0.27	*0.36	*0.46	*0.42	*0.33
		SEM	±0.04	±0.08	±0.09	±0.09	±0.10	±0.09
	Glycerol mMol/L	Mean	*0.061	*0.112	*0.144	*0.136	0.108	*0.076
		SEM	±0.022	±0.029	±0.032	±0.028	±0.022	±0.017
	Blood glucose mg/100 ml (n = 7)	Mean	4	11	*19	20	*20	*21
		SEM	±2	±5	±7	±7	±7	±8

*, * and * indicate a statistical significance of the changes from the pre stimulation level with P less than 0.05, 0.01 and 0.001 respectively

in most experiments (table I) and reached the maximal level about 2 minutes after the end of the stimulation (table II). In 9 dogs the mean level of FFA increased from 0.32 to 0.59 mEq/L (table I). The mean glycerol level increased from 0.088 to 0.160 mMol/L (tables I and II). The blood glucose concentration increased in some experiments (tables I and II). Five minutes after the start of the stimulation the mean heart rate and the mean blood pressure had increased from 137 to 181 beats per minute and from 127 to 169 mm Hg respectively (table I).

During stimulation in the *mesencephalic area* there were cardiovascular and metabolic changes qualitatively similar to those during the diencephalic stimulation (tables I and II). The mean FFA level increased from 0.24 to 0.78 mEq/L and the mean glycerol level from 0.082 to 0.258 mMol/L. There was also a statistically significant rise of the blood glucose level from 92 to 112 mg per 100 ml (tables I and II). The mean blood pressure and mean heart rate increased from 126 to 173 mm Hg and from 130 to 186 beats per minute respectively (table I).

The maximal individual changes in the levels of FFA and glycerol (table I) during stimulation in the *diencephalic* and the *mesencephalic area* were neither statistically correlated to the individual changes in blood presssure nor to the changes in heart rate

Muscle blood flow

Stimulation in the *diencephalic* and *mesencephalic* areas augmented the muscle blood flow *two to five times* The heart

TABLE I FFA and glycerol in arterial blood *plasma blood* glucose, and mean blood pressure and heart rate during 15 minute stimulations in the diencephalic and the mesencephalic areas. The figures give the individual mean *levels before the* stimulations (calculated on two determinations, one about 5 minutes before and the other immediately before) and the maximal levels during or 2 to 12 minutes after the stimulations

	Dog no	Voltage of stimulation	FFA mEq/l Before	FFA mEq/l Maximal	Glycerol mMol/l Before	Glycerol mMol/l Maximal	Blood glucose mg/100 ml Before	Blood glucose mg/100 ml Maximal	Mean blood pressure mm Hg Before	Mean blood pressure mm Hg Maximal	Heart rate beats/min Before	Heart rate beats/min Maximal
Diencephalic area	1	8	0 24	0 48	0 111	0 190	95	105	110	165	130	175
	2	10	0 38	0 96	0 097	0 153	95	100	125	140	175	190
	3	15	0 22	0 32	0 119	0 204	80	132	125	175	125	160
	4	10	0 39	0 91	0 071	0 174	70	82	165	235	90	130
	5	10	0 12	0 27	0 018	0 040	84	85	135	170	110	185
	6	8	0 24	0 45	0 147	0 265	100	102	120	140	175	225
	7	8	0 18	0 31	0 079	0 147	95	132	105	170	110	150
	8	8	0 48	0 83	0 042	0 114	93	115	125	170	150	225
	9	10	0 63	0 79	0 111	0 156	100	98	135	160	170	195
Mesencephalic area	1	8	0 30	1 12	0 106	0 326	102	104	100	150	120	175
	2	10	0 26	1 21	0 095	0 209	80	94	135	175	145	175
	3	10	0 12	0 48	0 059	0 241	130	190	125	175	125	210
	4	10	0 40	0 82	0 059	0 223	84	103	125	180	120	160
	5	10	0 13	0 34	0 030	0 158	76	95	125	165	125	220
	6	8	0 20	1 02	0 131	0 475	102	121	130	180	145	195
	10	8	0 25	0 49	0 130	0 236	74	83	110	150	130	170
	11	9	0 28	0 79	0 061	0 201	—	—	165	210	135	190

muscle area could be kept constant during rises of the systemic blood pressure The perfusion pressure and the muscle blood flow were recorded on a Grass polygraph (Model 5 C)

Analytical methods

The blood samples were immediately cooled in icewater Blood glucose was determined with the glucose-oxidase method, described by Marks (33) After centrifugation, plasma was immediately processed for determination of glycerol by the enzymatic method of Wieland (49) Plasma for analysis of FFA was frozen and stored during 1—5 days FFA were determined according to Dole s (13) method as modified by Trout et al (46) Control experiments showed that the freezing procedure did not influence the titration values All determinations were made in duplicate The statistical analyses were performed as recommended by Snedecor (42)

Results

FFA, glycerol, blood glucose, blood pressure and heart rate during stimulation in diencephalon and mesencephalon

During stimulation in the *diencephalic area* the plasma level of FFA increased

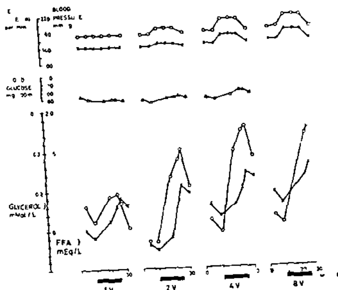


Fig 2 (Dog no 1 in table III) Arterial levels of FFA glycerol and blood glucose heart rate and mean blood pressure during stimulation in the *diencephalic area* The area was repeatedly stimulated during four 15-minute periods with varying voltages 1 2 4 and 8 V respectively as indicated by ▬

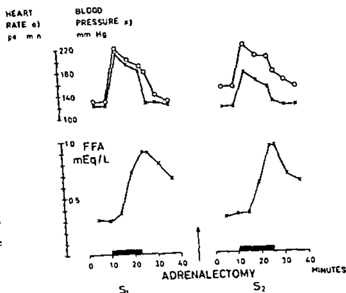


Fig 3 (Dog no 1 in table IV) Arterial level of FFA heart rate and mean blood pressure during stimulation in the *diencephalic area* before (S_1) and after (S_2) acute adrenalectomy

heart rate (dogs 1 and 2 in table III) In dog 1 stimulation with 1 V increased the level of FFA from 0 42 to 0 87 mEq/L and the glycerol level from 0 140 to 0 192 mMol/L the blood pressure and heart rate remaining unchanged (fig 2)

In dog 2 stimulation with 9 V increased the FFA level from 1 01 to

TABLE III FFA and glycerol in arterial blood plasma, blood glucose and mean blood pressure and heart rate during repeated 15 minute stimulations with varying voltages in the diencephalic and the mesencephalic areas The figure represent the individual mean levels before the stimulations and the maximal levels during or 2 to 12 minutes after the stimulations

Dog no	Area	Voltage	FFA mEq/L Before–Maximal	Glycerol mMol/L Before–Maximal	Blood glucose mg/100 ml Before–Maximal	Mean blood pressure mm Hg Before–Maximal	Heart rate beats/min Before–Maximal
1	Diencephalic	1	0 42–0 87	0 140–0 192	79– 80	140–140	170–170
		2	0 25–1 07	0 064–0 300	75– 83	140–145	170–185
		4	0 75–1 20	0 107–0 352	76– 95	145–160	170–200
		8	0 85–1 27	0 119–0 344	—	145–170	170–205
2	Diencephalic	9	1 01–1 61	0 195–0 303	130–158	150–150	160–160
		12	0 79–1 26	0 152–0 287	152–159	150–170	160–185
3	Diencephalic	3 5	0 15–0 17	0 053–0 055	67– 65	155–155	150–150
		7	0 12–0 28	0 055–0 096	65– 59	155–155	150–175
4	Diencephalic	1	0 35–0 39	0 037–0 053	70– 71	145–140	165–165
		3	0 49–0 62	0 083–0 089	70– 69	150–100	165–130
		9	0 43–0 50	0 061–0 089	72– 74	150–120	170–130
5	Mesencephalic	2 5	0 37–0 69	0 053–0 085	69– 73	160–155	150–150
		3 5	0 62 0 65	0 053–0 085	71– 72	160–160	155–165
		5 5	0 64–0 67	0 054–0 084	76– 74	160–180	155–180

rate always increased simultaneously When the blood pressure in the artery to the perfused muscle was kept constant by tightening the screw clamp, the blood flow was reduced during stimulation in some experiments This decrease was blocked by an i a injection of guanethidine (Ismelin®, Ciba) to the perfused muscles in a total dose of 2 mg This finding suggests an increased activity in the adrenergic vasoconstrictor nerves during stimulation In other experiments the increase in blood flow during stimulation was not inhibited by using the screw clamp, indicating the presence of an active vasodilatation In these dogs, i v administration of atropine in a dose of 0 2 mg/kg b w substantially reduced the increase in blood flow or produced a decreased flow This indicates an augmented activity in cholinergic vasodilator fibres (cf Uvnas (47) and Lindgren (32))

Stimulation with varying voltages

In two of five dogs there was a marked rise in the levels of FFA and glycerol without changes in blood pressure or

mean rises in blood pressure were from 137 to 188 mm Hg and from 115 to 172 mm Hg respectively The corresponding figures for the mean heart rate were from 153 to 194 and from 159 to 190 beats per minute (table IV)

Sympathetic ganglionic blockade

During the stimulation of the *diencephalic* (dogs 7 and 8) and *mesencephalic areas* (dogs 10 and 11) (table VI) prior to the administration of Agentit® the mean FFA level increased from 0 30 to 0 61 mEq/L The glycerol level increased from 0 078 to 0 175 mMol/L Five minutes after the start of the stimulation the mean rises in blood pressure and heart rate were from 126 to 175 mm Hg and from 131 to 184 beats per minute respectively

After the administration of the sympathetic blocking agent the FFA remained unchanged during stimulation (fig 4 and table VI) at a mean level of 0 24 mEq/L and glycerol at a mean level of 0 059 mMol/L The blood pressure tended to decrease from the initial mean level of 91 mm Hg The heart rate also tended to decrease from the initial mean frequency of 111 beats per minute

Examination of the brains

The localization of the electrode tips in the brains was easily recognized due to the coagulated spots

The brains from 16 experiments in which the *diencephalic area* had been stimulated were examined The coagulated spots were found in an area in the lateral and anterior part of the hypothalamus The area extended from just above the hypophysis and the mamillary bodies and up through the thalamus to the neighbourhood of the third ventricle The distance from the midline was 1 to 3 mm (fig 5)

The brains from 5 experiments in which the *mesencephalic* area had been stimulated were examined The coagulated spots were found to be concentrated in a rather limited area in the superior subcollicular gray matter 1—3 mm from the midline (fig 6)

The points in which the stimulations markedly increased the levels of FFA were scattered around in the two areas

and after acute adrenalectomy The figures are calculated on the individual changes from the mean of the mean

Stimulation after adrenalectomy					
5	10	15	17	22	27
−0 03	0 11	0 24	*0 34	*0 22	0 08
±0 02	±0 05	±0 09	±0 06	±0 06	±0 04

less than 0 05 0 01 and 0 001 respectively

TABLE IV FFA, mean blood pressure and heart rate during stimulation in the diencephalic area before and after acute adrenalectomy The figures give the individual mean levels before the stimulation and the maximal levels during or 2 to 12 minutes after the stimulations

Dog no	Voltage of stimulation	FFA mEq/L Before Adrenalectomy (Before–Maximal)	FFA mEq/L After Adrenalectomy (Before–Maximal)	Mean blood pressure, mm Hg Before Adrenalectomy (Before–Maximal)	Mean blood pressure, mm Hg After Adrenalectomy (Before–Maximal)	Heart rate, beats/min Before Adrenalectomy (Before–Maximal)	Heart rate, beats/min After Adrenalectomy (Before–Maximal)
1	8	0 31–0 90	0 34–0 94	135–215	115–175	130–220	150–220
2	10	0 62–1 09	0 29–0 51	150–190	125–225	165–185	150–185
3	9	0 74–1 04	0 29–0 64	115–175	100–150	150–185	150–175
4	9	0 18–0 49	0 26–0 47	150–200	125–165	155–185	155–180
5	8	0 25–0 63	0 28–0 52	150–175	125–175	165–220	180–210
6	10	0 32–0 83	0 53–1 01	120–170	100–140	155–170	170–170

1 61 mEq/L and the glycerol level from 0 195 to 0 303 mMol/L without change in blood pressure or heart rate

In the remaining three dogs, no or only small changes in FFA and glycerol were observed during stimulation even when significant changes in blood pressure and/or heart rate occurred

Adrenalectomy

Stimulation in the *diencephalic area* caused an increased arterial level of FFA even after adrenalectomy (fig 3, table IV and table V) The mean FFA rise before the adrenalectomy was from 0 40 to 0 83 mEq/L and after the adrenalectomy from 0 33 to 0 68 mEq/L The

TABLE V Changes in the arterial level of FFA during stimulation in the diencephalic area before concentration before the stimulations and give the mean values and the standard errors

Minutes after the start of the stimulations		Stimulation before adrenalectomy 5	10	15	17	22	27
FFA mEq/L	Mean	0 02	*0 26	*0 31	*0 41	*0 35	*0 24
	SEM	±0 02	±0 05	±0 06	±0 04	±0 06	±0 05

1, 2 and 3 indicate a statistical significance of the changes from the pre stimulation level with P

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The points in which the stimulations markedly increased the levels of FFA were scattered around in the two areas

and after acute adrenalectomy The figures are calculated on the individual changes from the mean of the mean

Stimulation after adrenalectomy					
5	10	15	17	22	27
−0 03 ±0 02	0 11 ±0 05	0 24 ±0 09	0 34 ±0 06	0 22 ±0 06	0 08 ±0 04

less than 0 05 0 01 and 0 001 respectively

TABLE IV FFA, mean blood pressure and heart rate during stimulation in the diencephalic area before and after acute adrenalectomy The figures give the individual mean levels before the stimulation and the maximal levels during or 2 to 12 minutes after the stimulations

Dog no	Voltage of stimulation	FFA mEq/L Before Adrenalectomy Before	Maximal	FFA mEq/L After Adrenalectomy Before	Maximal	Mean blood pressure mm Hg Before Adrenalectomy Before	Maximal	Mean blood pressure mm Hg After Adrenalectomy Before	Maximal	Heart rate beats/min Before Adrenalectomy Before	Maximal	Heart rate beats/min After Adrenalectomy Before	Maximal
1	8	0 31	0 90	0 34	0 94	135	215	115	175	130	220	150	220
2	10	0 62	1 09	0 29	0 51	150	190	125	225	165	185	150	185
3	9	0 74	1 04	0 29	0 64	115	175	100	150	150	185	150	175
4	9	0 18	0 49	0 26	0 47	150	200	125	165	155	185	155	180
5	8	0 25	0 63	0 28	0 52	150	175	125	175	165	220	180	210
6	10	0 32	0 82	0 53	1 01	120	170	100	140	155	170	170	170

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TABLE V Changes in the arterial level of FFA during stimulation in the diencephalic area before concentration before the stimulations and give the mean values and the standard errors

Minutes after the start of the stimulations		Stimulation before adrenalectomy 5	10	15	17	22	27
FFA mEq/L	Mean	0 02	[1]0 26	[2]0 31	[2]0 41	[2]0 35	[2]0 24
	SEM	±0 02	±0 05	±0 06	±0 04	±0 06	±0 05

[1], [2] and [3] indicate a statistical significance of the changes from the pre-stimulation level with P

pressure during 15-minute stimulations in the diencephalic and the mesencephalic areas before and the mean levels before the stimulations and the maximal levels during or 2 to 12 minutes after the

Glycerol mMol/L Before Maximal	Blood glucose mg/100 ml Before Maximal	Mean blood pressure mm *Hg* Before Maximal	Heart rate beats/min Before Maximal
0 079–0 147	95–132	105–170	110–150
0 070–0 066	88– 77	75– 60	100– 80
0 042–0 114	93–115	125–170	150–225
0 048–0 044	89– 85	75– 65	115–105
0 130–0 236	74– 83	110–150	130–170
0 050–0 037	71– 69	105–100	100– 95
0 061–0 201	–	165–210	135–190
0 0 0 0 065	85– 73	110– 75	130–125

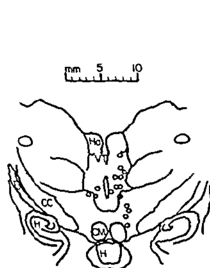


Fig 5 Schematic drawing showing the stimulation points from 16 experiments in which the *diencephalic area* was stimulated The drawing includes points 2 mm anterior and 2 mm posterior to the frontal plane shown in the figure CC = crus cerebri CM = corpus mamillare H = hypophysis, Ha = habenula Hi = hippocampus To = tractus opticus VT = ventriculus tertius

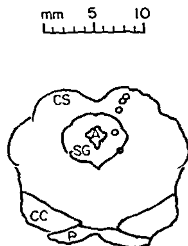


Fig 6 Schematic drawing showing the stimulation points from 5 experiments *in which* the *mesencephalic area* was stimulated The drawing includes points 1 mm anterior and 1 mm posterior to the frontal plane shown in the figures. A = aqueductus cerebri CC = crus cerebri CS = colliculus superior P = pons SG = substantia grisea centralis

TABLE VI FFA and glycerol in arterial blood plasma, blood glucose, heart rate and mean blood after administration of a sympathetic blocking agent (Agentit®) The figures give stimulations

Dog no	Area	Voltage			FFA mEq/L Before Maximal
7	Diencephalic	8	Before	Agentit®	0 18−0 31
			After		0 15−0 14
8	Diencephalic	8	Before	Agentit®	0.48−0 83
			After		0 18−0 16
10	Mesencephalic	8	Before	Agentit®	0 25−0 49
			After		0 19−0 16
11	Mesencephalic	9	Before	Agentit®	0 28−0 79
			After		0 45−0 48

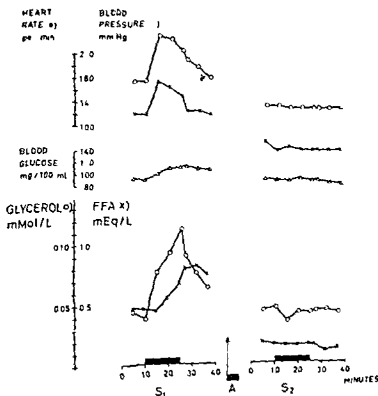


Fig 4 (Dog no 8 in tables I and VI) Arterial levels of FFA glycerol and blood glucose heart rate and mean blood pressure during stimulation in the diencephalic area before (S_1) and after (S_2) the administration of a sympathetic ganglionic blocking agent (Agentit®) (A)

Smith and co-workers (41), on stimulating small areas in the diencephalon in dogs (H_1 and H_2 fields of Forel), observed effects on left ventricular diameter, left ventricular pressure and heart rate, similar to those seen during exercise on a treadmill Later the same group (39) reported that even the pattern of peripheral blood flow seen during exercise was reproduced with remarkable accuracy by stimulation in the H_2 field of Forel

The *diencephalic area* studied in this investigation was situated dorsolateral to the mamillary bodies and was thus closely related to the H_1 and H_2 fields of Forel The effects on heart rate, blood pressure and muscle blood flow appear to be similar in nature to those reported by the Rushmer group (39)

Long term exercise is a stimulus which promotes the mobilization of FFA into plasma (15, 25) The arterial glycerol level and mobilization of glycerol into blood plasma also increase (10 25) When in this study higher intensities were used for the stimulation of the *diencephalic* and the *mesencephalic* areas the heart rate and blood pressure usually increased together with the levels of FFA and glycerol These stimulations may have activated neurons or pathways of importance for the circulatory as well as the metabolic adjustments to exercise

The possible role of the cholinergic vasodilator fibres for the circulatory changes in the early stages of muscular exercise has been discussed by Uvnas (47) In the present study there appeared to be no relation between the observed activation of vasodilator fibres and the rise in the FFA level during electrical supramedullary stimulation

The changes in the FFA level as well as the changes in heart rate and blood pressure were not abolished by adrenalectomy This finding is in agreement with Corell's observation (12) that electrical stimulation of hypothalamic structures produced a several fold rise of the FFA level, in intact as well as in adrenalectomized rabbits and monkeys The sympathetic ganglionic blocking agent Agentit[R] however here completely inhibited the metabolic and circulatory effects of the stimulations These findings suggest that the non adrenal part of the sympathetic nervous system may be of importance for the observed changes during supramedullary stimulation In this connection it is interesting to note that Basu et al (2) found no abnormality in the FFA metabolism during exercise in adrenalectomized patients maintained on cortisone

When lower intensities were used for electrical supramedullary stimulation in this study a rise in the levels of FFA and glycerol was observed without changes in heart rate or blood pressure These findings imply that the mobilization of FFA from adipose tissue is influenced by supramedullary structures, separate from neurons of importance for control of blood pressure and heart rate

Summary

The level of free fatty acids (FFA) in arterial blood plasma was followed during electrical stimulation in the diencephalon and the mesencephalon of anaesthetized dogs A significant rise of the FFA level was observed

Discussion

The arterial levels of free fatty acids and glycerol in blood plasma increased significantly during stimulation in two supramedullary regions which were located respectively in the hypothalamus and in the central gray matter in the mesencephalon

It has become evident that the adipose tissue is the main source for the plasma FFA in the fasting state (cf ref 26, 29, 48) The FFA are extracted from blood plasma by different organs such as the liver, heart muscle, skeletal muscles and the kidneys (17, 19, 28, 43) The utilization of FFA by the liver has been found to be proportional to the concentration of FFA in the medium surrounding the organ *in vitro* and to the concentration of FFA in the blood passing the organ *in vivo* (14, 34, 38) In the myocardium and in the skeletal muscles the uptake of FFA from the blood similarly depends on the arterial FFA level (40, 44)

Variations in the blood flow through different organs also influence the efflux of FFA from blood plasma For example, when the blood flow to the isolated perfused rat liver was increased there was an augmented total uptake of FFA (35) With respect to the FFA uptake in the skeletal muscles Carlsson and Pernow (6) found that in man the FFA concentration in the femoral artery was lower than in the femoral vein during rest, suggesting a FFA release from adipose tissue During exercise, however, the FFA level in the vein became lower than in the artery, indicating a removal of FFA from blood plasma The arterial levels of FFA also decreased during short periods of exercise There was a significant linear relationship between the decrease in FFA and the increase in heart rate (7, 8) When Spitzer and co-workers (28, 44) electrically stimulated isolated skeletal muscles there was an increased blood flow through the muscles and the total extraction of FFA from the blood increased The extraction ratio decreased

Glycerol is released from the adipose tissue when triglycerides are hydrolyzed during the lipolysis (cf 4, 9, 11) In contrast to FFA, glycerol is probably not utilized by adipose tissue or by skeletal muscles The glycerol level in blood plasma has therefore been used as an indicator of lipolytic activity in the adipose tissue (9, 10, 21) The liver is the organ of major importance for the removal of glycerol from blood plasma (4, 27, 36) Thus the efflux of glycerol from blood plasma may be affected by changes in the liver blood flow but not by changes in the muscle blood flow

Studies *in vivo* with isotopes have shown that generally, changes in the plasma FFA concentration are brought about by variations in the FFA production rate (1, 11, 26, 45) It has also been found that the increased glycerol level in arterial plasma during infusion of noradrenaline, for example, is mainly due to an increased mobilization of glycerol into plasma (9, 24) From these facts it appears likely that the rise in the levels of FFA and glycerol in blood plasma, seen in this study during supramedullary stimulation was due to an enhanced rate of mobilization of FFA and glycerol, caused by an increased lipolysis in adipose tissue

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during stimulation in two areas One was located dorsolateral to the mamillary bodies, 1—3 mm from the mid line in the diencephalon, and the other 6 mm further back in the subcollicular mesencephalic gray matter The level of glycerol in blood plasma always increased together with the FFA level The stimulations in the mesencephalic area caused a significant rise in the blood-glucose level There was an increase in blood pressure and heart rate during stimulation in the two areas The effects on FFA, blood pressure and heart rate were still present after adrenalectomy but were abolished after treatment with a sympathetic ganglionic blocking agent

Stimulations with varying voltages showed that it was possible to produce a rise of the levels of FFA and glycerol without changes in heart rate or blood pressure The results suggest that the mobilization of FFA from adipose tissue into plasma is influenced by supramedullary structures separate from the vasomotor neurons The significance of supramedullary structures for metabolic and cardiovascular adjustments to exercise is discussed

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From the Departments of Medicine (Head P Bechgaard, M D), Surgery (Head P Holm-Nielsen M D) and Diagnostic Radiology (Head Aa Wagner M D) Aarhus Amtssygehus, Denmark

Pseudo-ulcer and True Peptic Ulcer

A Clinical, Radiographic and Statistical Follow up Study

By

EINAR KRAG

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The pseudo ulcer syndrome which is defined on the basis of clinical and radiographic criteria has been known for a long time and the term is more or less synonymous with such diagnoses as gastroduodenitis pylorogastritis gastritis etc Several authors (Torben Andersen 2) Lykke Olesen (11 12) Ostrow and Resnick (15)) have shown that clinically it is not possible to distinguish between gastroduodenitis (pylorogastritis) and peptic ulcer Barium meal examination will afford the decisive criteria Rivers 16 of the Mayo Clinic reported on 191 patients with pronounced symptoms of "ulcer disease Subsequent operation revealed that in slightly less than one half of the cases no ulcer was present

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TABLE I Survey of patients treated for 'ulcer disease' in the Department of Medicine, Aarhus Amtssygehus, in 1936—1945

	No of cases
Non bleeding pseudo-ulcer	174
Non bleeding gastric ulcer	51
Non bleeding duodenal ulcer	205
Non bleeding pyloric ulcer	13
Bleeding gastric ulcer	7
Bleeding duodenal ulcer	46
Bleeding pyloric ulcer	4
Combined ulcers	21
Haematemesis melaena but no ulcer	52
Total	573

patients with pseudo-ulcer were characterized by (a) a perfectly normal radiographic picture (apart from hypotonicity), and (b) the 'pyloric triad," i e (1) periodically occurring cardialgia, (2) hunger pain, and (3) relief of pain afforded by food However, the two last-mentioned symptoms were sometimes replaced by gastric haemorrhage, nocturnal gastric distress or vomiting of copious amounts of gastric juice

Barfred did not find any difference in the age distribution of the patients with pseudo ulcer and those with peptic ulcer *During the observation period,* stenosis and perforation did not occur *among* the patients with pseudo ulcer None of the patients was subjected to gastric operations Manifest bleeding was not as frequent as in patients with peptic ulcer The follow up study showed that peptic ulcer had developed in 74 of the 235 patients with pseudo ulcer, i e 31 % Barfred concluded that pseudo-ulcer is not a clinical entity, but, on the other hand, it is not merely a stage in the development of peptic ulcer It represents a less severe course of the ulcer disease" The only way in which the two varieties of the disease can be differentiated is to subject the patients to repeated radiographic examinations

Material and methods

During the years 1936—1945 inclusive, a total of 174 patients was discharged from the Department of Medicine, Aarhus Amtssygehus, with the diagnoses of gastroduodenitis, pylorogastritis or gastritis (The diagnoses were clinical in the sense that they were not based on histological or gastroscopic studies of the gastric mucosa) All the patients had been subjected to barium meal examination which had however failed to reveal peptic ulcer in any of them The following symptoms were *characteristic of the patients* (1) Cardialgia of a few weeks' duration alternating with periods in which this symptom was absent (2) hunger pain and (3) relief of pain afforded by food In some of the patients *this triad was not very distinct* and one of the symptoms was sometimes replaced by nocturnal gastric distress, pyrosis, or sharply localized epigastric pain (pinpoint pain)

From this series of patients, who have previously been studied (11 12) all cases in which the dyspeptic complaints might be referable to some other disease (e g cholelithiasis) were excluded and so were the patients who had suffered from manifest gastric bleeding on admission All the patients were subjected to the same diagnostic and therapeutic measures in the department *Usually, they had been on a restricted diet* for four weeks.

The 174 patients constitute a homogenous group all suffering from ulcer like dyspepsia but without radiographically demonstrable ulcer

During the same period 205 patients with non bleeding duodenal ulcer and 51

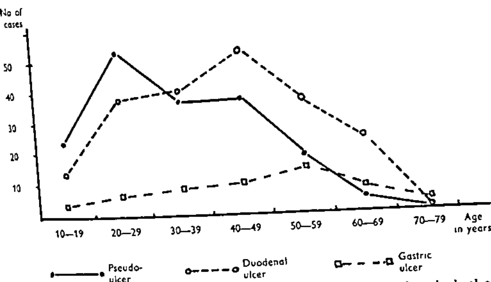


Fig 1 Age distribution of patients with non bleeding pseudo-ulcer non bleeding duodenal or gastric ulcer on first admission in 1936—1945

with non bleeding gastric ulcer were treated in the department Of all the patients admitted with ulcer disease those with pseudo-ulcer represent about 30 % (table I)

FIRST ADMISSION DURING THE PERIOD 1936—1945

Age distribution The average age of the pseudo-ulcer patients was significantly lower ($p < 0.001$) than that of the patients with either duodenal or gastric ulcer (fig 1) This is not in agreement with Barfred s study in which no age difference was revealed

Sex distribution Table II shows that there was a male preponderance among the patients with pseudo-ulcer There was no significant difference between the male to-female ratios in pseudo-ulcer and duodenal ulcer whereas such a difference was revealed between pseudo-ulcer and gastric ulcer (p 0 05 Barfred found that women were relatively more predominant among patients with pseudo-ulcer in which the male to-female ratio was 2 8 1 as compared with 4 8 1 in peptic ulcer

Duration of symptoms This was on the whole shorter for patients with pseudo-ulcer The difference in duration between these patients and those with duodenal ulcer was significant ($p < 0.01$) whereas this was not the case between pseudo-ulcer and gastric ulcer (table III) It is seen from the table that a large proportion of the series consisted of chronic cases with a duration of symptoms of more than 5 years

Gastric acidity As was customary during the period under consideration gastric acidity was determined by Ewald s test meal

TABLE II Sex distribution at first admission in 1936—1945

	Men	Women	Total	Sex ratio M/F
Pseudo-ulcer	138	36	174	3 8 1
Duodenal ulcer	156	49	205	3 2 1
Gastric ulcer	33	18	51	1 8 1

TABLE I Survey of patients treated for "ulcer disease" in the Department of Medicine, Aarhus Amtssygehus, in 1936—1945

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The 174 patients constitute a homogenous group all suffering from ulcer like dyspepsia, but without radiographically demonstrable ulcer

During the same period 205 patients with non bleeding duodenal ulcer and 51

TABLE IV Incidence of subsequent development of true ulcer (follow up examination 1963) in 174 patients with non bleeding pseudo-ulcer at the first admission in 1936—1945

	Men		Women		Total	
	No	%	No	%	No	%
Follow up examination 1963						
Ulcer had developed	54	39	15	42	69	40
Ulcer had not developed	56	41	15	42	71	41
No definite information	28	20	6	16	34	19
Total	138	100	36	100	174	100

FOLLOW UP STUDIES

In 1963, the patients were subjected to a follow up study Information was obtained of 92 % of the pseudo-ulcer patients, whereas 8 % (i e 14 patients) could not be traced or had emigrated Apart from one case all the patients with duodenal and gastric ulcer were followed up

All the patients who had died or been subjected to gastric operation were followed to death or operation Those who were alive and had not undergone gastric operation were requested to return to the hospital for an interview and barium meal examination

As regards the patients who had died before the follow up examination informa tion as to the cause of death and the course of the ulcer disease was obtained in each individual case by means of death certificates and through the patients own doctors

The hospital records for all patients who had been admitted to hospital for medical or surgical treatment of the ulcer disease during the observation period were reviewed

Results

Does true ulcer subsequently develop in pseudo ulcer patients?

As appears from table IV the pseudo ulcer patients may be divided into three groups on the basis of the results of the follow up examination viz (a) one in which ulcer was demonstrated either during the observation period or at the follow up (40 %), (b) another in which radiography gastric operation or autopsy performed during the observation period failed to reveal an ulcer (41 %) and (c) a third group consisting of an appreciable number of patients who had not been subjected to diagnostic stomach examinations after the first admission during 1936—1945 (19 %) Most of the patients in the last group had died during the observation period It is known that in these patients the gastric disease clinically ran a quiet course and did not give rise to admission to hospital but obviously it cannot be excluded that ulcer may nevertheless have developed in some of them

It is seen from table IV that among the 174 patients who were treated for pseudo ulcer during the period 1936—1945 true ulcer had developed in 40 % at the follow up examination in 1963 In view of the aforementioned statements this turn-over frequency must be regarded as a minimum figure

The question may then be asked How great is the risk of acquiring ulcer

TABLE III Duration of symptoms at the first admission in the period 1936—1945

Duration of symptoms (years)	Pseudo-ulcer		Duodenal ulcer		Gastric ulcer	
	(No)	(%)	(No)	(%)	(No)	(%)
<1/2	28	16	19	9	12	23
1/2—2	31	18	35	17	5	10
2—5	44	25	35	17	8	16
>5	71	41	116	57	26	51
Total	174	100	205	100	51	100

Definitions Ewald s test was performed as follows

The patient was given 35 g rusk and 250 ml water in the morning on a fasting stomach Exactly 1 hour later the gastric contents were aspirated Gastric lavage with 300 ml water was then performed, and the remainder of the gastric contents was calculated *The total gastric contents* were considered to be equal to the aspirated amount plus the calculated remainder *The total gastric acidity* (the phenolphthalein index) was expressed by the number of 0 1 ml of 0 1 N NaOH required to neutralize 10 ml of the gastric contents (indicator phenolphthalein)

Acid secretion (*Kemp's index*)

$$\frac{\text{Phenolphthalein index} \times \text{total gastric contents}}{100}$$

In other words Kemp s index gives a measure of the total amount of acid secreted expressed in millilitres of 0 1 N HCl

In Ewald s test meal Kemp (8) fixed the limit between normal acid secretion and increased digestive acid secretion at 100 ml of 0 1 N HCl

Reproducibility In the series under consideration Ewald s test meal was performed in duplicate Statistical analysis of these duplicate determinations showed that only in about 75 % of the patients were both determinations on the same side of the upper limit of normal i e 100 ml of 0 1 N HCl *Thus the results obtained by Ewald s test meal are only to a limited extent reproducible*

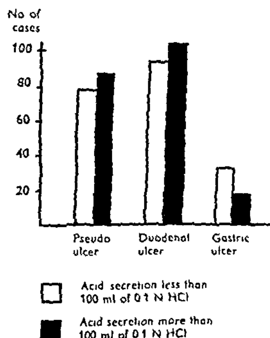


Fig 2 Gastric acidity determined by Ewald s test meal during the first hospital stay in 1936—1945

It appears from fig 2 that the acid secretion differed only slightly in patients with pseudo-ulcer and those with peptic ulcer and that the numbers of cases below and above 100 ml of 0 1 N HCl were nearly equal in the two groups Thus, pseudo-ulcer cannot be differentiated from peptic ulcer on the basis of determinations of gastric acidity by Ewald s test

TABLE V Comparison of the ulcer incidence in patients with pseudo-ulcer and the total population served by Aarhus Amtssygehus

Sex	Case material	No of individuals	Ulcer frequency	
			No of cases	%
M	Total population	36 300	[1] 8 300	23
	Pseudo-ulcer patients	138	54	39
F	Total population	35 800	[1] 5 400	15
	Pseudo-ulcer patients	36	15	42

[1] The ulcer incidence was calculated on the basis of Watkinson s figures from Leeds

TABLE VI The male to-female ratio in peptic ulcer and "turn-over" pseudo-ulcers

Sex ratio	The period 1936—1945		"Turn-over" pseudo-ulcers 1963	
	Gastric ulcer	Duodenal ulcer	Gastric ulcer	Duodenal ulcer
M/F	1 8 1	3 2 1	2 0 1	3 6 1

tion differs from that in pseudo-ulcer patients In table V Watkinson s figures have been applied to the group of the population (17) which was served by Aarhus Amtssygehus in the period 1936—1945

On the basis of these figures it may be concluded that *development of true ulcer is significantly more frequent in patients with pseudo ulcer than in the general population* ($p<0.001$)

For the pseudo-ulcer patients in whom true ulcer subsequently developed the male to-female ratio is shown in table VI There is no significant difference in this sex ratio between the "turn-over" pseudo-ulcer patients and the patients who were admitted with true ulcer during the period 1936—1945 This is in contrast with the results reported by Barfred, who found that the male to-female ratio was lower in "turn over" pseudo ulcer patients than in patients in whom ulcer was revealed at the first examination

Table VII shows the relationship between duodenal and gastric ulcers both in patients who originally had peptic ulcer in the period 1936—1945 and in those with "turn-over pseudo ulcers at the follow up examination in 1963 It is seen that juxtapyloric ulcers are more predominant in the "turn over" ulcers This may perhaps be taken to suggest that pseudo-ulcer is an intermediary link in the production of a juxtapyloric ulcer However, statistically there is no significant difference

during a lifetime in the general population?

Two Danish investigators, Alsted (1) and J L Hansen (6) have performed detailed studies on the ulcer incidence Alsted's investigation, which was based on questionnaires sent to all Danish doctors in 1940 and 1948, provided information as to the ulcer incidence in a certain month From the data obtained Alsted estimated that ulcer is present in 18 % of the men and 6 % of the women of the adult population over 20 years J L Hansen subjected a series of 2,239 autopsy cases from the period 1937—1946 to a statistical analysis He found that ulcer is almost equally frequent in men and women, the average frequencies being 4 49 and 4 01 %, respectively The highest frequency for men was 6 24 % (age group 80—89 years) and for women 7 02 % (age group 80—89) Fatal ulcers were not included in these figures

Levij (10) studied a series of 8,974 autopsied cases from the years 1940—1957 He found an average incidence of 23 5 % in persons over 20 years of age, but this figure also included fatal ulcers The frequency of chronic, non-fatal ulcers was highest in men in the age group 50—59 years (20 4 %) and in women over 80 years (15 3 %) However, as J L Hansen pointed out, a retrospective analysis of cases which have been autopsied by various pathologists is attended with great difficulties

In Leeds, Watkinson (18) performed a very careful pathological study on the ulcer incidence among autopsy cases During the period 1930—1949, all autopsies, i e 13,000, in the Pathology Department of the University of Leeds were performed under the personal supervision of Professor Matthew Stewart, who was specifically on the look-out for ulcers and ulcer scars, irrespective of the cause of death On the basis of "ulcers found incidentally" among the autopsy cases, i e after the exclusion of the highly selected group of ulcer deaths, the average incidence in men was found to be 14 2 % and in women 7 7 % The highest incidence in men was found in the age group 45—65 years (23 %) and in women in the age group 65—75 years (15 %)

Watkinson's study must be assumed to be the one which gives the most reliable expression of ulcer incidence among the general population As his results, at least as far as men are concerned, are in good agreement with Alsted's figures, it may be justifiable to regard them as being applicable to the Danish population

In a comparison of the ulcer incidence in Watkinson's series and the 174 patients with pseudo ulcer considered here, the most correct procedure would obviously be to compare the individual age groups in the two series, but as the present series is too small for such a division a comparison is instead made for each sex between the age group in the Leeds series which shows the highest ulcer incidence and the average ulcer incidence in the pseudo ulcer patients When Watkinson's results are applied in this way to the Danish population, the resultant ulcer incidence will possibly be too high, but it is difficult to find a better expression in the present analysis, the purpose of which is to study if the ulcer incidence in the general popula-

in the ratio of juxtapyloric to corpus ulcers in the two groups

In which pseudo-ulcer patients will true ulcer subsequently develop?

In order to throw light on this question it was studied whether a number of factors considered on the first admission in the period 1936—1945 were significantly related to subsequent development of true ulcer

The following alternative variables were studied
Sex Male v female
Age Under v over 40 years
Gastric acidity Secretion below v above 100 ml 0 1 N HCl
Duration of symptoms Less than v more than 2 years
Radiographic findings Normal condition v coarse, irregular mucosal folds
For detailed information as to the statistic methods used the reader is referred to Moroney (14)

A variance analysis showed that the age of first admission is of importance since the development of true ulcer was much more frequent in patients over 40 than in those under that age (table VIII) The difference is significant ($p<0.01$) The other factors studied were of no significance in the subsequent development of ulcer in the pseudo-ulcer patients

Prognosis of pseudo ulcer related to that of duodenal and gastric ulcers

The prognosis was studied by dividing the series into three main groups according to the clinical course favourable, less favourable and serious An attempt was made to make this classification as objective as possible by means of the following definitions

Group A Favourable course

1 Complete freedom of symptoms throughout the observation period after the first admission in the period 1936—1945
2 Not symptom free during the entire period after the first admission but the gastric disease did not cause re admission bleeding or absence from work Stomach medicine or diet was sometimes necessary but both remedies were not used at the same time

ulcers at follow up in 1963

Duodenal ulcer						Gastric ulcer					
Males		Females		Total		Males		Females		Total	
No	%	No	%	No	%	No	%	No	%	No	%
40	26	17	35	57	28	9	27	9	50	18	35
23	15	11	23	34	17	7	21	4	22	11	22
93	9	20	42	113	55	17	52	5	28	22	43
1[illegible]	100	48	100	204	100	33	100	18	100	51	100
0		1		1		0		0		0	
156		49		205		33		18		51	

TABLE VII Ratio of various ulcer types in patients with peptic ulcer and "turn over" pseudo-ulcer

	Sex	Duodenal ulcers / Gastric ulcers	Juxtapyloric ulcers / Corpus ulcers = Duodenal + pyloric ulcers / Gastric ulcers
Peptic ulcers from the period 1936–1945	M	156/33 = 4.7:1	165/33 = 5.0:1
	F	49/18 = 2.7:1	53/18 = 2.9:1
"Turn over" pseudo-ulcers in 1963	M	36/6 = 6.0:1	46/6 = 7.3:1
	F	10/3 = 3.3:1	12/3 = 4.0:1

TABLE VIII Prognostic significance of age in the development of true ulcer in patients with non bleeding pseudo-ulcer at first admission

	Age of patients at first admission			
	Under 40 years		Over 40 years	
	No	%	No	%
Ulcer developed	35	31	34	56
Ulcer did not develop	61	54	10	16
No definite information	17	15	17	28
Total	113	100	61	100

TABLE IX Clinical prognosis of non bleeding pseudo-ulcer and non bleeding duodenal and gastric

	Pseudo-ulcer					
	Males		Females		Total	
Clinical course	No	%	No	%	No	%
A Favourable	61	48	16	49	77	48
B Less favourable	26	20	9	27	35	22
C Serious	40	32	8	24	48	30
Total followed up	127	100	33	100	160	100
Not followed up	11		3		14	
Total	138		36		174	

in the ratio of juxtapyloric to corpus ulcers in the two groups

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2 Not symptom free during the entire period after the first admission but the gastric disease did not cause re admission bleeding or absence from work Stomach medicine or diet was sometimes necessary, but both remedies were not used at the same time

ulcers at follow up in 1963

Duodenal ulcer						Gastric ulcer					
Males		Females		Total		Males		Females		Total	
No	%	No	%	No	%	No	%	No	%	No	%
40	26	17	35	57	28	9	27	[illegible]	50	18	35
[illegible]3	15	11	23	34	17	7	21	4	22	11	22
93	59	20	42	113	55	17	52		28	22	43
156	100	48	100	204	100	33	100	18	100	51	100
0		1		1		0		0		0	
156		49		205		33		18		51	

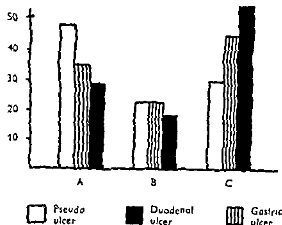


Fig 3 Clinical prognosis of non bleeding pseudo ulcer and peptic ulcer in 1963, expressed in relative figures A Favourable, B less favourable, C serious

Group B Less favourable course

3 One manifest gastric bleeding during the observation period
4 One re admission for medical treatment of the gastric disease
5 One or more flare ups of the gastric disease without re-admission or bleeding The disease caused absence from work and/or the use of medicine and diet

Group C Serious course

6 Two or more manifest gastric bleedings during the observation period
7 Two or more re-admissions to hospital for medical treatment of the gastric disease during the observation period
8 Suture of perforated ulcer performed during the observation period
9 Gastric operation for the 'ulcer disease' (apart from suture of perforated ulcer) during the observation period
10 Death from the 'ulcer disease

The prognosis of non bleeding pseudo ulcer, non bleeding duodenal ulcer and non-bleeding gastric ulcer appears from table IX and is expressed in relative figures in fig 3

Statistical analysis of the figures in table IX showed that pseudo ulcer has a definitely better prognosis than duodenal ulcer The difference is significant ($p < 0.001$) On the other hand, pseudo ulcer and gastric ulcer showed no significant difference in prognosis

A similar analysis of *the frequency of gastric operation* in patients with pseudo ulcer, gastric ulcer and duodenal ulcer revealed that this frequency was significantly lower in pseudo ulcer than in duodenal ulcer ($p < 0.001$), whereas there was no difference between pseudo ulcer and gastric ulcer (table X)

The indications for operation in the pseudo ulcer patients appear from table XI In about three quarters of the cases operation was performed because of

TABLE X Frequency of gastric operations in patients with non bleeding pseudo ulcer or peptic ulcer

	Pseudo ulcer					
	Males		Females		Total	
	No	%	No	%	No	%
Gastric operation	18	14	4	12	22	14
No gastric operation	109	86	29	88	138	86
Total followed up	127	100	33	100	160	100

TABLE XI Indications for operation in patients with pseudo-ulcer

	Haematemesis melaena	Pyloric stenosis	Incapacity for work medical treatment ineffective
Men	2	2	14
Women	0	2	2
Total	2	4	16

persistent dyspepsia, incapacity for work and lack of effect of the medical treatment

Manifest bleeding occurred in 9 % of the pseudo-ulcer patients during the observation period Table XII shows that manifest bleeding was more frequent in peptic ulcer Here only the difference between pseudo-ulcer and duodenal ulcer was significant ($p < 0.02$)

Which factors are of prognostic significance?

It was found to be of interest to study statistically if some of the factors considered on the first admission in the period 1936–1945 were of prognostic significance in patients with non bleeding pseudo-ulcer non bleeding duodenal ulcer and non bleeding gastric ulcer These factors were a sex (b age (c) duration of symptoms, and (d) gastric acidity The statistical methods applied were those described in the section on the ulcer incidence in pseudo-ulcer patients

Variance analysis showed that *the duration of symptoms* is of decisive importance in the prognosis for pseudo ulcer patients It is seen from table XIII that patients who had had symptoms for more than 2 years have a poorer prognosis than those with a shorter duration of symptoms The difference is significant ($p < 0.01$) Similar results ($p < 0.01$) were obtained in gastric and duodenal ulcer (table XIV) As far as gastric and duodenal ulcers were concerned the prognosis was poorer *in men* than in women (table XV) The difference is significant ($p < 0.01$) In pseudo-

d[illegible] ring the observation period

Duodenal ulcer						Gastric ulcer					
Males		Females		Total		Males		Females		Total	
No		No		No		No		No	%	No	
[illegible]	4[illegible]	10	[illegible]1	82	40	10	30	2	11	12	24
84	4[illegible]	38	[illegible]	122	[illegible]0	[illegible]3	[illegible]0	16	83	39	[illegible]6
1[illegible]	100	48	100	[illegible]04	100	33	100	18	100	51	100

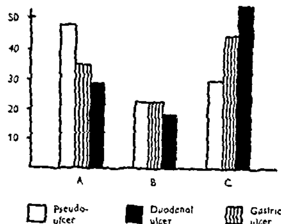


Fig 3 Clinical prognosis of non bleeding pseudo-ulcer and peptic ulcer in 1963, expressed in relative figures A Favourable, B less favourable C serious.

Group B Less favourable course

3 One manifest gastric bleeding during the observation period
4 One re admission for medical treatment of the gastric disease
5 One or more flare ups of the gastric disease without re admission or bleeding The disease caused absence from work and/or the use of medicine and diet

Group C Serious course

6 Two or more manifest gastric bleedings during the observation period
7 Two or more re-admissions to hospital for medical treatment of the gastric disease during the observation period
8 Suture of perforated ulcer performed during the observation period
9 Gastric operation for the 'ulcer disease' (apart from suture of perforated ulcer) during the observation period
10 Death from the 'ulcer disease'

The prognosis of non bleeding pseudo ulcer, non-bleeding duodenal ulcer and non-bleeding gastric ulcer appears from table IX and is expressed in relative figures in fig 3

Statistical analysis of the figures in table IX showed that pseudo-ulcer has a definitely better prognosis than duodenal ulcer The difference is significant ($p < 0.001$) On the other hand, pseudo ulcer and gastric ulcer showed no significant difference in prognosis

A similar analysis of *the frequency of gastric operation* in patients with pseudo ulcer, gastric ulcer and duodenal ulcer revealed that this frequency was significantly lower in pseudo ulcer than in duodenal ulcer ($p < 0.001$), whereas there was no difference between pseudo ulcer and gastric ulcer (table X)

The indications for operation in the pseudo ulcer patients appear from table XI In about three quarters of the cases operation was performed because of

TABLE X Frequency of gastric operations in patients with non bleeding pseudo-ulcer or peptic ulcer

	Pseudo-ulcer					
	Males		Females		Total	
	No	%	No	%	No	%
Gastric operation	18	14	4	12	22	14
No gastric operation	109	86	29	88	138	86
Total followed up	127	100	33	100	160	100

Duodenal ulcer						Gastric ulcer					
Males		Females		Total		Males		Females		Total	
No	%	No	%	No	%	No	%	No	%	No	%
34	22	2	4	36	18	5	15	1	6	6	12
122	78	46	96	168	82	28	85	17	94	45	88
156	100	48	100	204	100	33	100	18	100	51	100

TABLE XV Prognostic significance of sex in patients with non bleeding peptic ulcer

	Gastric ulcer				Duodenal ulcer			
	Men		Women		Men		Women	
Clinical course	No	%	No	%	No	%	No	%
A Favourable	9	27	9	50	40	26	17	35
B Less favourable	7	21	4	22	23	15	11	23
C Serious	17	52	5	28	93	59	20	42
Total followed up	33	100	18	100	156	100	48	100

TABLE XVI The relationship between the frequency of gastric operations and the radiographic appearance of the mucosal pattern in patients with non bleeding pseudo-ulcer on first admission in 1936—1945

Radiographic appearance of gastric mucosa		Gastric operations		Total of patients with pseudo-ulcer	
		No	%		
Coarse irregular mucosal folds	Men	11	22	50	
	Women	3	23	13	
	Total	14	22		63
Normal conditions	Men	7	8	88	
	Women	1	4	23	
	Total	8	7		111
				Total	174

TABLE XII *The frequency of haematemesis and/or melaena during the observation period*

	Pseudo-ulcer					
	Males		Females		Total	
	No	%	No	%	No	%
Bleeding	11	9	3	9	14	9
No bleeding	116	91	30	91	146	91
Total followed up	127	100	33	100	160	100

TABLE XIII Prognostic significance of the duration of symptoms in patients with non bleeding pseudo ulcer

	Men				Women			
	Duration of symptoms				Duration of symptoms			
	< 2 years		> 2 years		< 2 years		> 2 years	
Clinical course	No	%	No	%	No	%	No	%
A Favourable	23	51	38	46	8	80	8	35
B Less favourable	15	33	11	13	2	20	7	30
C Serious	7	16	33	41	0	0	8	35
Total followed up	45	100	82	100	10	100	23	100

TABLE XIV Prognostic significance of the duration of symptoms in patients with non bleeding peptic ulcer

	Gastric ulcer				Duodenal ulcer			
	Duration of symptoms				Duration of symptoms			
	<2 years		>2 years		2 years		>2 years	
Clinical course	No	%	No	%	No	%	No	%
A Favourable	8	47	10	29	18	33	39	26
B Less favourable	1	6	10	29	13	24	21	14
C Serious	8	47	14	42	23	43	90	60
Total followed up	17	100	34	100	54	100	150	100

Duodenal ulcer						Gastric ulcer					
Males		Females		Total		Males		Females		Total	
No	%	No	%	No	%	No	%	No	%	No	%
34	22	2	4	36	18	5	15	1	6	6	12
122	78	46	96	168	82	28	85	17	94	45	88
156	100	48	100	204	100	33	100	18	100	51	100

TABLE XV Prognostic significance of sex in patients with non bleeding peptic ulcer

	Gastric ulcer				Duodenal ulcer			
	Men		Women		Men		Women	
Clinical course	No	%	No	%	No	%	No	%
A Favourable	9	27	9	50	40	26	17	35
B Less favourable	7	21	4	22	23	15	11	23
C. Serious	17	52	5	28	93	59	20	42
Total followed up	33	100	18	100	156	100	48	100

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Radiographic appearance of gastric mucosa		Gastric operations		Total of patients with pseudo-ulcer	
		No	%		
Coarse irregular mucosal folds	Men	11	22	50	
	Women	3	23	13	
	Total	14	22		63
Normal conditions	Men	7	8	88	
	Women	1	4	23	
	Total	8	7		111
				Total	174

ulcer there was no difference between the two sexes. None of the other factors considered were of any prognostic significance. It should specifically be noted that this also applied to gastric acidity.

Prognostic significance of mucosal pattern on the radiograph

As already mentioned, barium-meal examination in the 174 patients with pseudo ulcer on the first admission in the period 1936—1945 failed to reveal true ulcer. Coarse, irregular mucosal folds were seen in 63 cases, while the mucosal pattern was perfectly normal in 111.

As is seen from table XVI, the number of gastric operations was significantly higher in patients with coarse, irregular mucosal folds than in those who had a normal mucosal pattern ($p < 0.01$). The difference is still significant even if it is assumed that the radiographic diagnosis of coarse, irregular folds is beset with an uncertainty of $\pm$ 20 %.

Discussion

As appears from the definition of pseudo ulcer the radiographic appearance is the crucial diagnostic criterion in the differentiation of pseudo ulcer from peptic ulcer.

The radiographic diagnoses in the present series were made by the same radiologist both in the period from 1936 to 1945 and at the follow up examination in 1963. During the period under consideration no decisive changes have ocurred in the technique of radiographic diagnosis of gastric lesions. There is thus no reason to assume that it should be easier today to reveal any hidden ulcers in the 174 patients with pseudo-ulcer than it was in the period 1936—1945. Unfortunately, only relatively few of the films from the period 1936—1945 were available in 1963, but in the study in 1946 (11, 12) the chief radiologist subjected all the barium meal radiographs to a careful reappraisal.

The aforementioned coarse, irregular folds of the gastric mucosa, which seem to bear a certain relationship to the frequency of gastric operations, were only recorded when the radiologist found that these changes were unquestionable.

During the observation period (17—27 years), pseudo ulcer ran a milder clinical course than did peptic ulcer. Several of the significance tests revealed a difference only between pseudo ulcer and duodenal ulcer. However, there is nevertheless a distinct difference in the relative figures (percentages) between pseudo ulcer and gastric ulcer in most of the tables, but because of the small absolute figures in the gastric ulcer group, it is not possible to demonstrate significant differences between that group and pseudo ulcer.

The differences between Barfred's study and the present analysis (age and sex distribution) may probably be explained by the different methods applied in the selection of two series. Barfred's cases were selected among both in- and out patients, and cases with manifest bleeding were included. The present series was selected among patients admitted to hospital, and only patients without manifest bleeding were included.

On essential points, agreement exists between Barfred's series and the present analysis, both show a marked tendency among pseudo-ulcer patients to subsequent development of true ulcer, and a better clinical prognosis of pseudo ulcer than of peptic ulcer

The statistical analysis of the factors which may exert an influence on the prognosis showed that a long duration of symptoms results in a poorer prognosis both in pseudo-ulcer and peptic ulcer As far as the latter condition is concerned, this is a well known fact On the other hand, it is worthy of note that gastric acidity is without prognostic importance both in pseudo-ulcer and peptic ulcer In the variance analyses, in which it is possible to isolate the influence of a single factor, it appeared that gastric acidity was the factor which exerted the slightest influence on the prognosis

Summary

A clinical and radiographic follow up study was performed on 174 patients with non bleeding pseudo ulcer 205 patients with non bleeding duodenal ulcer and 51 patients with non bleeding gastric ulcer after an observation period of 17—27 years Of the patients concerned 92 % with pseudo ulcer and 100 % of those with duodenal or gastric ulcer were traced The main purpose of the study was to throw light on the natural history and prognosis of the pseudo ulcer syndrome including its relationship to duodenal and gastric ulcer

1 A diagnosis of pseudo-ulcer was made when symptoms of peptic ulcer (pyloric syndrome) were present although radiography failed to reveal any ulcer lesion
2 Pseudo-ulcer is a common syndrome and is present in about 30 % of the patients exhibiting symptoms of 'ulcer disease'
3 Patients with pseudo ulcer are younger than those with peptic ulcer
4 Men are more predominant among patients with pseudo-ulcer than those with peptic ulcer
5 The duration of symptoms is shorter in patients with pseudo-ulcer than in those with peptic ulcer
6 Gastric acidity does not differ in pseudo ulcer and peptic ulcer
7 Peptic ulcer subsequently develops in about 40 % of the patients with pseudo ulcer This frequency is significantly higher than in the general population
8 Peptic ulcer develops especially in pseudo ulcer patients over 40 years
9 The clinical course of pseudo-ulcer is significantly milder than that of peptic ulcer and the frequencies of gastric operation and manifest bleeding in the former are significantly lower than in the latter condition
10 A duration of symptoms exceeding 2 years signifies a poorer prognosis both in pseudo ulcer and in duodenal and gastric ulcer
11 Gastric acidity is of no prognostic importance in pseudo-ulcer and peptic ulcer
12 In pseudo-ulcer patients the frequency of gastric operation is higher among those in whom barium meal examination reveals coarse

irregular mucosal folds than in the remainder

In recapitulation, it may be said that pseudo-ulcer represents a special course of the "ulcer disease" with a better prognosis Even though true ulcer subsequently develops in an appreciable number of the patients with pseudo-ulcer, it will scarcely be correct to regard pseudo ulcer as a precursor of peptic ulcer In all probability, it will be more correct to conceive the syndrome as an independent disease entity

Acknowledgement

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From the Geriatric Unit, Old People's Town Copenhagen and the Hormone Department, Statens Seruminstitut, Copenhagen Denmark

A Study on the Urinary Excretion of 17-ketogenic Steroids in a Group of Geriatric Patients on Different Diets

By

Torben Geill, Svend G Johnsen and Bent Mackeprang

The causes of atheromatosis still remain unelucidated, but a number of factors have been found to play an aetiological role heredity hypertension chronic infection mental stress excessive cigarette smoking insufficient physical activity, hormonal balance and nutrition

Apart from local metabolic factors in the arterial wall an injury to the vessel wall mechanical or chemical must be considered as the starting point of atheromatosis In addition, there are depositions of cholesterol and other lipids due to disturbances of the fat metabolism Hormones in particular thyroid hormone and the sex hormones influence the lipid metabolism and presumably thereby also atheromatosis This last mentioned view is supported by therapeutic experiments using the named hormones

A much-discussed question during recent years is whether the dietary content of hormonal substances is able to influence the development of atheromatosis It has been suggested that the rare occurrence of coronary disease among Bantu Negroes is due to given factors in particular the nature of their diet (2 11), a brisk physical activity and possibly the high incidence of hepatic diseases The excretion of oestrogens and of 17 ketosteroids was studied by several authors (1 2 3 4 5 7, 10)

The influence of diet upon blood coagulation and fibrinolysis has been discussed in recent years but the results are far from consistent To some extent the discrepancy must be considered a consequence of differences in analytical methods A recent study in the Old People's Town (Geriatric Unit), Copenhagen (9) showed increased thrombin formation determined by the thrombin generation test in Ollendorff's modification (8) in the lipaemic phase on a diet rich in butter fat and reversely a reduced thrombin formation in the lipaemic phases on a diet rich in vegetable oil or fish oil In connection with these investigations the present

Submitted for publication May 6 1965

irregular mucosal folds than in the remainder

In recapitulation, it may be said that pseudo-ulcer represents a special course of the "ulcer disease" with a better prognosis Even though true ulcer subsequently develops in an appreciable number of the patients with pseudo-ulcer, it will scarcely be correct to regard pseudo-ulcer as a precursor of peptic ulcer In all probability, it will be more correct to conceive the syndrome as an independent disease entity

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Mannitol VI	Normal I VII	Normal II VIII	Total	Obs	$\bar{x}$	Normal: No of obs	Normal: Common mean value
8.9	10 0	9 3	93 1	8	11 6	4	10 0
59	6 0	4 3	37 8	8	4 7	4	5 1
13 5	7 8	9 4	61 0	8	7 6	4	6 8
5 4	4 2	4 4	49 8	8	6 2	5	5 9
8.2	5 5	4 8	53 8	8	6 7	4	5 9
6.5	c	8 0	44 8	7	6 4	3	7 1
4 4	b—	–	27 6	6	4 6	2	3 5
2 7	5 2	3 9	20 0	5	4 0	3	4 4
5 7	5 3	(7 4)	28 5	5	5 7	2	5 1
61 2	44 0	51 3	–	–	–	–	–
9	7	8	–	–	–		–
6 8	6 3	6 4	–	–	–	–	–
b—			26 3	5	5 3	2	5 4
8.9	10 2	7 9	76 4	8	9 6	5	10 2
(9 1)	6 4	7 7	56 2	8	7 0	5	6 9
6 9	9 5	9 4	73 7	8	9 2	5	6 8
6 7	10 1	7 7	52 6	8	6 6	4	6 8
3 6	5 2	c—	28 3	6	4 7	3	5 3
9 4	9 4	(13 2)	82 1	8	10 3	4	8 3
8.2	9 9	4 5	78 8	8	9 9	5	9 1
–		–	26 2	4	6 6	2	5 5
3 7	6 0	6 1	47 9	8	6 0	5	5 6
6 4	7 0	6 6	56 6	8	7 1	5	6 9
6 3	8 8	8.3	65 5	8	8 2	5	8.7
b—		–	37 3	5	7 5	3	6 9
12 4	9 6	(4 5)	85 8	8	10 7	4	12 0
8 8	95 9	75 9		–	–	–	–
11	11	10				–	–
7 2	8 7	7 6				–	
141 0	133 3	127 2	–				
0	18	18					
7 0	7 8	7 1	–				–

c collection of urine failed ( ) = urinary output suspicious cf text

TABLE I Urinary excretion of 17 KGS, measured in mg/24 hours

No	Age	Dietary periods: Normal I	High fat II	Normal III	Vegetable oil IV	Normal V
Females						
4	77	(6.4)	10.0	9.5	27.5	11.5
5	78	4.6	4.2	6.0	4.8	(3.0)
10	84	5.9	8.0	(3.2)	8.3	4.9
11	87	5.7	6.8	7.8	7.1	8.4
12	89	5.6	4.0	(10.1)	7.6	8.0
17	77	(4.4)	5.3	7.6	7.0	6.0
18	75	4.3	4.1	2.9	(9.9)	(2.0)
22	78	a—	—	—	4.1	4.1
23	76	a—	—	—	5.1	5.0
Total		36.9	42.4	46.1	81.4	52.9
No of obs		7	7	7	9	9
Mean value		5.3	6.1	6.6	9.0	5.9
Males						
7	77	(6.4)	5.1	4.8	4.1	5.9
1	75	11.4	8.0	12.6	8.1	9.3
2	94	7.8	6.4	7.0	6.0	5.8
3	82	11.2	11.2	9.8	6.7	9.0
6	88	(3.1)	8.7	5.7	6.0	4.6
8	94	6.5	5.6	4.9	c—	(2.5)
9	76	8.3	(16.8)	9.7	9.1	6.2
13	80	12.0	10.7	11.0	11.7	10.8
14	82	6.7	7.5	4.4	7.6	b—
15	83	6.3	(12.0)	4.0	3.6	6.2
16	87	5.9	6.5	7.7	9.1	7.4
19	79	8.7	5.5	10.0	9.7	8.2
20	86	6.8	9.7	7.5	7.0	6.3
21	83	11.1	11.8	14.2	8.5	13.7
Total		112.2	125.5	113.3	97.2	95.9
No of obs		14	14	14	13	13
Mean value		8.0	9.0	8.1	7.5	7.4
Males and females						
Total		149.1	167.9	159.4	178.6	148.8
No of obs		21	21	21	22	22
Mean value		7.1	8.0	7.6	8.1	6.8

a = not included in the study until the stated period b = excluded from the experiment

ſanne d I	Normal I VII	Normal II VIII	To al	Obs	$\bar{X}$	Normal: No of obs	Normal: Geom mean value
89	10 0	9 3	93 1	8	11 6	4	10 0
9	6 0	4 3	37 8	8	4 7	4	5 1
13 5	7 8	9 4	61 0	8	7 6	4	6 8
5 4	4 2	4 4	49 8	8	6 2	5	5 9
8 2	5 5	4 8	53 8	8	6 7	4	5 9
6.5	c	8 0	44 8	7	6 4	3	7 1
4 4	b—		27 6	6	4 6	2	3 5
2 7	5 2	3 9	20 0	5	4 0	3	4 4
5 7	5 3	(7 4)	28 5	5	5 7	2	5 1
61 2	44 0	51 3					
9	7	8					
6 8	6 3	6 4					
b			26 3	ɔ	5 3	2	5 4
8 9	10 2	7 9	76 4	8	9 6	5	10 2
(9 1)	6 4	7 7	ɔ6 2	8	7 0	5	6 9
6 9	9 5	9 4	73 7	8	9 2	5	6 8
6 7	10 1	7 7	52 6	8	6 6	4	6 8
3 6	5 2	c—	28 3	6	4 7	3	5 5
9 4	9 4	(13 2)	82 1	8	10 3	4	8 3
8 2	9 9	4 5	78 8	8	9 9	5	9 1
			26 2	4	6 6	2	5 5
3 7	6 0	6 1	47 9	8	6 0	5	5 6
6 4	7 0	6 6	56 6	8	7 1	5	6 9
6 3	8 8	8 3	65 5	8	8 2	5	8 7
b—			37 3	5	7 5	3	6 9
12.4	9 6	(4 5)	85 8	8	10 7	4	12 0
8 8	95 9	75 9					
11	11	10					
7 2	8 7	7 6					
140 0	139 9	127 2					
0	18	18					
7 0	7 8	7 1					

c collection of urine fail d ( )=urinary output susp ous cf ex

TABLE I Urinary excretion of 17 KGS, measured in mg/24 hours

No	Age	Dietary periods: Normal I	High fat II	Normal III	Vegetable oil IV	Normal V
Females						
4	77	(6.4)	10.0	9.5	27.5	11.5
5	78	4.6	4.2	6.0	4.8	(3.0)
10	84	5.9	8.0	(3.2)	8.3	4.9
11	87	5.7	6.8	7.8	7.1	8.4
12	89	5.6	4.0	(10.1)	7.6	8.0
17	77	(4.4)	5.3	7.6	7.0	6.0
18	75	4.3	4.1	2.9	(9.9)	(2.0)
22	78	a—	—	—	4.1	4.1
23	76	a—	—	—	5.1	5.0
Total		36.9	42.4	46.1	81.4	52.9
No of obs		7	7	7	9	9
Mean value		5.3	6.1	6.6	9.0	5.9
Males						
7	77	(6.4)	5.1	4.8	4.1	5.9
1	75	11.4	8.0	12.6	8.1	9.5
2	94	7.8	6.4	7.0	6.0	5.8
3	82	11.2	11.2	9.8	6.7	9.0
6	88	(3.1)	8.7	5.7	6.0	4.6
8	94	6.5	5.6	4.9	c —	(2.5)
9	76	8.3	(16.8)	9.7	9.1	6.2
13	80	12.0	10.7	11.0	11.7	10.8
14	82	6.7	7.5	4.4	7.6	b—
15	83	6.3	(12.0)	4.0	3.6	6.2
16	87	5.9	6.5	7.7	9.1	7.4
19	79	8.7	5.5	10.0	9.7	8.2
20	86	6.8	9.7	7.5	7.8	6.3
21	83	11.1	11.8	14.2	8.5	13.7
Total		112.2	125.5	113.3	97.2	95.9
No of obs		14	14	14	13	13
Mean value		8.0	9.0	8.1	7.5	7.4
Males and females						
Total		149.1	167.9	159.4	178.6	148.8
No of obs.		21	21	21	22	22
Mean value		7.1	8.0	7.6	8.1	6.8

a = not included in the study until the stated period b – excluded from the experiment

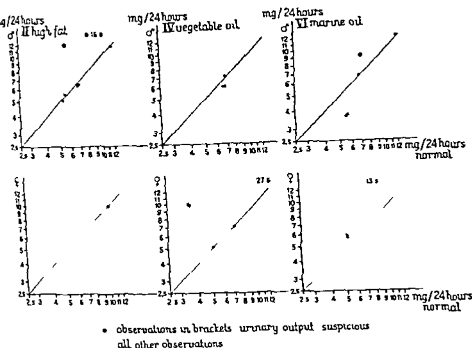


Fig 1 Comparison of 17 KGS excretion during various periods on an altered diet with the corresponding values for periods on normal diet

2 Comparison of 24 hour excretion of 17 KGS towards the end of the periods on normal diet

This comparison was done by calculating the geometric mean for each period These mean values include only observations which are not in brackets Thereafter the results for the individual periods were compared with the mean There was no difference between the 5 normal periods The marked divergences were predominantly among persons in brackets i e the divergences might be due to errors in collecting the urines

3 Comparison of 24 hour 17-KGS excretion towards the end of periods on an altered diet with the corresponding values for periods on a normal diet

In fig 1 the 17 KGS excretion for the various periods is plotted the mean values for the normal periods for males and females separately It will be seen that most divergences are small and non systematic Among major divergences the majority are of the type having a urinary output differing in the same direction

authors also determined the 24 hour excretion of 17 ketogenic steroids This aspect of the study will be reported below

tal periods, and sent to the Hormone Department, Statens Seruminstitut, where the content of 17 ketogenic steroids (17 KGS) was determined by the so-called Norymberski II technique in the modification of Jorgensen (6)

Material and methods

The material comprised 23 patients from one of the nursing departments of the geriatric hospital (Old People s Town) in Copenhagen During the period of the study, which was nearly one year, 5 patients died The youngest patient was 73 and the oldest 92 years of age Periods with normal diet alternated with diets high in butter fats, vegetable oils (soya bean oil, cotton seed oil) and marine oils (fish oil, seal oil) respectively The duration of the normal periods was 5—12 weeks, of the butter fat period 5 weeks, of the vegetable oil period 6 weeks and of the marine oil period 4 weeks

The hospital diet given during the normal periods had a fat content of approximately 80 g, mostly in the form of butter fat and margarine and a caloric content of 2,000—2,500

In the vegetable oil period butter and ordinary margarine were replaced by a factory made medical margarine containing 65 % unhydrogenated cotton seed oil, and milk was replaced by soya bean oil emulsified in skimmed milk

During the marine oil period only fish, mostly fish like salmon eel, herring mackerel and fat Greenlandic halibut (hippoglossus vulgaris) were given for lunch and supper instead of meat A highly refined seal oil (iodine value approx 190) was used partly for cooking and partly for a hospital made margarine with 80 % unhydrogenated seal oil This seal oil had very little taste and smell, and the patients accepted it without any objections

24 hour urines were collected from all the subjects towards the end of all the periods the normal periods as well as the experimen

Results

Table I gives the urinary excretion of 17-ketogenic steroids in *mg per 24 hours* within the various periods There is no positive correlation between the dietary content of saturated fatty acids and the urinary excretion of 17-ketogenic steroids

The results presented in this table were assessed in collaboration with Mr Michael Weiss Bentzon, actuary, Department of Statistics, Statens Seruminstitut, by the following comparisons

1 Comparison of 17-KGS excretion and urine output

For each patient a diagram was traced, in which the 24 hour excretion of 17-KGS is the ordinate and the urinary output the abscissa In many cases there is no relationship between the two quantities Among the remaining patients there are a few showing divergences in the same direction for ordinate and abscissa For instance, the urinary output may be considerably lower (higher) than the average and the 17 KGS at the same time low (high) Presupposing that errors occur in the collection of the 24 hour urine it appears reasonable to be particularly suspicious of observations of this type (given in brackets in the table)

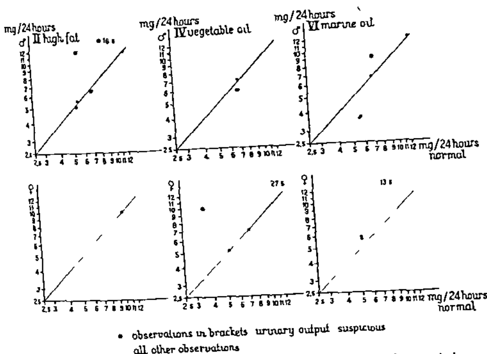


Fig 1 Comparison of 17 KGS excretion during various periods on an altered diet with the corresponding values for periods on normal diet

2 Comparison of 24 hour excretion of 17 KGS towards the end of the periods on normal diet

This comparison was done by calculating the geometric mean for each period These mean values include only observations which are not in brackets Thereafter the results for the individual periods were compared with the mean There was no difference between the 5 normal periods The marked divergences were predominantly among persons in brackets i e the divergences might be due to errors in collecting the urines

3 Comparison of 24 hour 17 KGS excretion towards the end of periods on an altered diet with the corresponding values for periods on a normal diet

In fig 1 the 17 KGS excretion for the various periods is plotted the mean values for the normal periods for males and females separately It will be seen that most divergences are small and non systematic Among major divergences the majority are of the type having a urinary output differing in the same direction

Summary

Twenty-three geriatric patients (14 males and 9 females) were put on a dietary regimen in which periods on normal diet alternated with periods on a diet having a varying content of saturated and unsaturated fatty acids respectively

The 24-hour urinary excretion of 17-ketogenic steroids, determined towards the end of the various dietary periods, showed no positive correlation with the degree of saturation of the fatty acids used in the diets

Acknowledgement

This investigation was supported by a grant from the F L Smidth Jubilee Foundation

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Acta Medica Scandinavica Vol 178, fasc 6, 1965

From the Department of Medicine (Head C A Hernberg M D), Maria Hospital Helsinki Finland

Comparison of Acetohexamide with Other Sulfonylurea Compounds in the Treatment of Diabetes Mellitus

By

Bo Berglund and Theodor Jakobson

Acetohexamide (Eli Lilly Co) is an oral antidiabetic drug of the sulfonylurea group which is structurally related to both tolbutamide and chlorpropamide It differs from tolbutamide in the substitution of an acetyl group in the para position of the phenyl ring and the incorporation of a cyclohexyl group on the urea portion of the chain fig 1)

The hypoglycemic effect of acetohexamide has been well documented in clinical trials (1 3 14, 15 16 17) The mode of action of this oral drug has been reported to be similar to the earlier sulfonylurea compounds and is probably dependent upon the functional integrity of the pancreatic islets for effectiveness The duration of action of acetohexamide is reported to be from 12 to 24 hours and permits the use of only one daily dose 13 23)

Animal experiments (15) as well as pharmacological studies in humans (18) seem to indicate that acetohexamide is essentially non toxic and more effective than tolbutamide and since so-called secondary failures are frequently encountered with the use of the latter drug (4, 6) treatment with acetohexamide would seem to be indicated in these cases

Acetohexamide has, on the other hand been reported to be less effective than chlorpropamide (17, 19) while comparisons of the effect of acetohexamide and carbutamide have so far not been reported

The purpose of this paper is to report our experience with acetohexamide in diabetic patients who have previously been maintained with other sulfonyl urea compounds or with a combination of sulfonylurea and phenylethylbiguanide (DBI) The investigation has been designed as a short term study in which the previously used sulfonylurea drugs have been replaced by equivalent amounts of acetohexamide during one or several short periods of treatment in an effort to evaluate the effectiveness of acetohexamide as compared with the other oral agents

Submitted for publication May 14 1965

Summary

Twenty-three geriatric patients (14 males and 9 females) were put on a dietary regimen in which periods on normal diet alternated with periods on a diet having a varying content of saturated and unsaturated fatty acids respectively

The 24-hour urinary excretion of 17-ketogenic steroids, determined towards the end of the various dietary periods, showed no positive correlation with the degree of saturation of the fatty acids used in the diets

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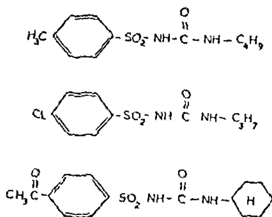


Fig 1 Structural formulae for acetohexamide (bottom), chlorpropamide (middle) and tolbutamide (top)

Material and methods

The material consists of a total of 63 adult-onset diabetics who were treated at the out-patient department of the Maria Hospital in Helsinki The patients were divided into five different groups according to the previous treatment which consisted of tolbutamide carbutamide or chlorpropamide administered either alone or in combination with DBI The age of the patients (mean 65 years, range 49 to 79 years) and the known duration of diabetes (mean 4 9 years range 2 months to 12 years) was similar in all groups The number of the patients belonging to the different groups as well as the duration of treatment with the various oral agents can be seen in table I

Treatment with acetohexamide was instituted in these patients during scheduled visits to the out patient department by replacing the previously used sulfonylurea compound with acetohexamide The patients who had previously been treated with tolbutamide or carbutamide received the same amount of acetohexamide and in the patients in whom the previous treatment consisted of chlorpropamide every 0 25 g tablet was replaced by 0 5 g of acetohexamide In the diabetic patients receiving combined treatment with sulfonylurea drugs and DBI the sulfonlyurea compound was similarly replaced by acetohexamide without changing the amount of DBI

The patients received during the treatment with the oral drugs a sugar free diet with a carbohydrate content not in excess of 200 g per day but a quantitative calculation of the diet was as a rule not carried out No special adjustments of the diet were made after the addition of acetohexamide to the diabetic regimen

Determination of blood glucose with a glucose oxidase method (9) and polariscopic estimations of urinary sugar were carried out following the commencement of acetohexamide therapy during one or several periods of treatment varying in length from two weeks to three months After every period the patient was switched back again to the same oral agent which had been used prior to the period of treatment with acetohexamide without making any changes in the previously used dosage of the drug Out of the 63 diabetic patients in whom treatment with acetohexamide was originally initiated 43 patients received acetohexamide during one period of treatment while two periods of treatment were used in 15 cases and in 5 cases the drug was administered during three different periods of treatment between which the patient was each time switched back to the originally used oral treatment During the periods of treatment with acetohexamide and during treatment with the other oral drugs an effort was made to record blood glucose and urinary glucose excretion at least two or three times respectively and 9 cases in which the criteria for diabetic control had been checked only once were later excluded from the material The combined length of the periods of treatment with acetohexamide and the average dose of the drug as well as the duration of treatment and dosage of the other oral agents are recorded in table I

Results

The average values for blood glucose and the excretion of urinary glucose in the above mentioned groups of diabetic patients following treatment with aceto

TABLE I Average dose and duration of previously used oral treatment and subsequent acetohexamide therapy in different groups of diabetic patients

Group	No of pat.	Average duration of previous treatment (years)	Duration of treatment and dosage of drugs during present study			
			Acetohexamide		Other sulfonylurea drugs	
			Mean dose	Duration of treatment (weeks)	Mean dose	Duration of treatment (weeks)
I Tolbutamide	18	2.4	0.86 (0.5–1.5) g	9	0.86 (0.5–1.5) g	10
II Carbutamide	10	4.[illegible]	0.875 (0.5–1.5) g	9	0.875 (0.5–1.25) g	13
III Chlorpropamide	10	2.9	0.89 (0.5–1.25) g	11	0.445 (0.25–0.625) g	12
IV Tolbutamide + DBI	9	1.5	1.14 (1.0–1.5) g + 57 (25–100) mg	12	1.14 (1.0–1.5) g + 57 (25–100) mg	10
V Chlorpropamide + DBI	7	1.7	0.91 (0.5–1.0) g + 70 (25–100) mg	9	0.455 (0.25–0.50) g + 70 (25–100) mg	9

hexamide and following treatment with other hypoglycemic oral drugs are recorded in table II It can be seen that a significant decrease of blood glucose values (P < 0.02) and a highly significant decrease of the excretion of urinary glucose (P < 0.001) could be noted following replacement of tolbutamide by acetohexamide and a significant decrease of blood glucose levels (P < 0.01) and the excretion of urinary glucose (P < 0.02) could likewise be seen in the patients who prior to the acetohexamide treatment had received a combination of tolbutamide and DBI

In the patients in whom the previous treatment had consisted of carbutamide no appreciable change of diabetic control could be noted following replacement of the previously used drug by acetohexamide while a significant increase of glycosuria (P < 0.05) was noted in the diabetic patients who were switched over from chlorpropamide to acetohexamide In the patients in whom the previous treatment had consisted of a combination of chlorpropamide and DBI a small but insignificant decrease of glycosuria was observed following replacement of chlorpropamide by acetohexamide

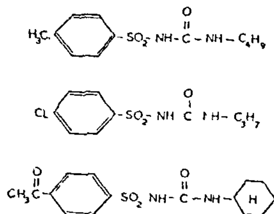


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The patients received during the treatment with the oral drugs a sugar free diet with a carbohydrate content not in excess of 200 g per day but a quantitative calculation of the diet was as a rule not carried out. No special adjustments of the diet were made after the addition of acetohexamide to the diabetic regimen.

Determination of blood glucose with a glucose oxidase method (9) and polariscopic estimations of urinary sugar were carried out following the commencement of acetohexamide therapy during one or several periods of treatment varying in length from two weeks to three months. After every period the patient was switched back again to the same oral agent which had been used prior to the period of treatment with acetohexamide without making any changes in the previously used dosage of the drug. Out of the 63 diabetic patients in whom treatment with acetohexamide was originally initiated 43 patients received acetohexamide during one period of treatment while two periods of treatment were used in 15 cases and in 5 cases the drug was administered during three different periods of treatment between which the patient was each time switched back to the originally used oral treatment. During the periods of treatment with acetohexamide and during treatment with the other oral drugs an effort was made to record blood glucose and urinary glucose excretion at least two or three times respectively and 9 cases in which the criteria for diabetic control had been checked only once were later excluded from the material. The combined length of the periods of treatment with acetohexamide and the average dose of the drug as well as the duration of treatment and dosage of the other oral agents are recorded in table I.

Results

The average values for blood glucose and the excretion of urinary glucose in the above mentioned groups of diabetic patients following treatment with aceto-

patients following treatment with acetohexamide and other oral drugs

Urinary glucose					
Other sulfonylurea drugs		Acetohexamide			
Mean	S D	Mean	S D	t	P
0 6	20 9	13 3	17 2	4 33	<0 001
21 8	15 7	27 5	29 0	1 19	
40 0	33 2	56 3	40 9	2 88	<0 05
60 4	44 2	40 0	27 4	3 15	<0 02
46 0	44 0	45 7	34 3	0 03	

similarly recorded in fig 3 The patients who received DBI in addition to chlorpropamide are in this figure likewise marked with filled circles It can be seen that although an impairment of diabetic control in terms of an increase of glycosuria was noted in most cases following replacement of chlorpropamide by acetohexamide a decrease of glycosuria was observed in a few cases belonging to the group in which DBI was used as an adjunct to the sulfonylurea treatment

The number of the diabetic patients in whom diabetic control according to commonly used criteria could be regarded as good following short term treatment with acetohexamide and following treatment with other oral antidiabetic drugs respectively can be seen in table III Blood glucose values under 200 mg per cent and an excretion of urinary glucose not in excess of 20 g were considered in these patients to be consistent with good diabetic control A marked increase of the number of well controlled cases following treatment with acetohexamide can be noted in the diabetic patients in whom the previous treatment consisted of tolbutamide alone or in combination with DBI while the number

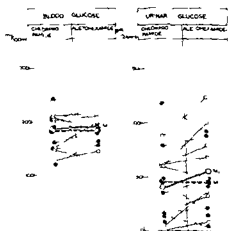


Fig 3 Changes of blood glucose levels and urinary glucose excretion following replacement of chlorpropamide with acetohexamide The open circles represent values observed in diabetic patients treated with sulfonylurea drugs alone while the cases which at the same time received DBI as an adjunct to the sulfonylurea therapy are indicated by filled circles
M_1 = Mean values of the sulfonylurea group
M = Mean values of the group in which the treatment consisted of sulfonylurea + DBI

TABLE II Comparison of mean values of blood glucose and urinary glucose excretion in diabetic

Group	No of pat	Blood glucose					
		Other sulfonylurea drugs		Acetohexamide			
		Mean	S D [1]	Mean	S D [1]	t	P
I Tolbutamide	18	189 0	56 6	159 2	30 0	2 84	< 0 02
II Carbutamide	10	169 1	55 5	169 7	34 1	0 06	
III Chlorpropamide	10	187 6	35 8	192 6	46 5	0 52	
IV Tolbutamide+DBI	9	194 6	35 5	166 4	24 5	4 08	< 0 01
V Chlorpropamide + DBI	7	183 9	41 5	185 1	20 8	0 11	

[1] S D = Standard deviation

The blood glucose levels and the values for urinary glucose excretion of the individual diabetic patients belonging to groups I and IV are seen in fig 2

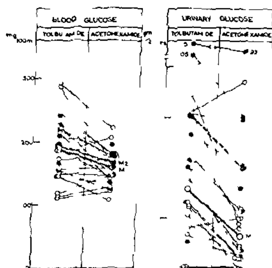


Fig 2 Changes of blood glucose levels and urinary glucose excretion following replacement of tolbutamide with acetohexamide The open circles represent values observed in diabetic patients treated with sulfonylurea drugs alone while the cases which at the same time received DBI as an adjunct to the sulfonylurea therapy are indicated by filled circles M_1 = Mean values of the sulfonylurea group M_2 = Mean values of the group in which the treatment consisted of sulfonylurea + DBI

Most of the recorded values for both blood and urinary glucose are average values of two or three single determinations It should be noted that in the majority of the cases in which tolbutamide was replaced by acetohexamide improvement of diabetic control can be seen although some cases did not meet generally accepted criteria of "good control" following treatment with acetohexamide and a few additional patients did apparently not benefit at all from the change of therapy The cases who received combined treatment with DBI are marked with filled circles which are combined with broken lines At least some improvement of blood glucose levels and glycosuria was noted in most cases following replacement of tolbutamide by acetohexamide, although diabetic control in this group of patients was generally less good than in the tolbutamide group

The blood glucose levels and the values for the excretion of urinary glucose of the individual diabetic patients belonging to groups III and V are

acetohexamide This would seem to imply that the hypoglycemic effect of both oral agents is of a similar magnitude and is in accordance with the known stronger hypoglycemic effect of carbutamide as compared with tolbutamide

The results of the present investigation suggest on the other hand that acetohexamide is on an average less effective than chlorpropamide This is in accordance with other clinical observations (3, 16 17 19) It has been claimed, however that patients who fail to react to *one* sulfonylurea drug do occasionally respond to another generally less potent hypoglycemic agent (5) and also chlorpropamide failures have thus been reported to respond to acetohexamide (3) Although chlorpropamide undoubtedly is usually a more potent hypoglycemic agent serious toxic manifestations such as cholestatic jaundice (20) are occasionally encountered with its use and chlorpropamide is for this reason regarded by many authors to be definitely more toxic than tolbutamide which seldom causes any complications of this kind

Clinical experience with the use of acetohexamide is to date less extensive than which chlorpropamide Available clinical reports suggest however that toxic manifestations are very seldom encountered Kirtley et al reported thus only 2 cases of jaundice out of 1 500 cases receiving acetohexamide treatment (12) Montgomery et al on the other hand have reported elevated levels of alkaline phosphatase in diabetic patients who had received acetohexamide (16) It has been pointed out however that abnormalities of liver function in diabetic patients must be interpreted with caution since abnormal tests are frequently encountered in diabetics who ha*ve not been on any oral* drugs (14)

It has already been mentioned, that the duration of action of acetohexamide is longer than that of tolbutamide (5, 15) and acetohexamide can therefore be used in one daily dose in contrast to the latter drug which must be administered in divided dosage The duration of the hypoglycemic effect produced by acetohexamide seems to be intermediate between that of tolbutamide and chlorpropamide but the duration of action is *nevertheless considerably shorter than* with the use of the latter drug which has been reported to have a half life of 36 hours in body fluids in its active form and a duration of action extending up to 60 hours (8 13) Hypoglycemic effects can consequently be expected to occur less frequently in patients receiving acetohexamide than with the use of chlorpropamide and the absence of hypoglycemia during treatment with acetohexamide has in effect been pointed out (7)

During the course of the present study symptoms attributable to hypoglycemia were noted only in one case Other subjective side effects were likewise remarkably few except for a few instances of slight gastrointestinal intolerance which was almost invariably encountered in the diabetic patients who at the same time received treatment with DBI and could probably in most case be attributed to the latter drug

The results of the combined treatment with acetohexamide and DBI are encouraging in so far as this combination of

TABLE III Number of patients in whom good diabetic control was achieved following administration of acetohexamide and following treatment with other oral drugs

Previous treatment	Good control following treatment with the previously used oral drugs	Good control following treatment with acetohexamide
Tolbutamide	7/18	14/18
Carbutamide	4/10	4/10
Chlorpropamide	4/10	3/10
Tolbutamide + DBI	2/9	4/9
Chlorpropamide + DBI	2/7	2/7

of diabetics with "good" control decreased by one following replacement of chlorpropamide by acetohexamide and remained unchanged in the other groups

Very few signs of intolerance were encountered in the patients who received treatment with acetohexamide Hypoglycemic symptoms were noted in one patient while anorexia, nausea or slight diarrhea were observed in four additional cases Three of these patients, however, received DBI in addition to acetohexamide Three patients had slight proteinuria which in every case had been present already before the initiation of acetohexamide therapy Liver function tests and leucocyte counts were, except in a few cases, not carried out during the course of the present study

Discussion

Acetohexamide was during the present investigation found to be significantly more effective than tolbutamide in reducing blood glucose levels and glycosuria in adult onset diabetic patients This finding is consistent with other clinical reports on the effectiveness of acetohexamide (3, 16, 17, 19) Secondary failure to oral diabetic agents and in particular tolbutamide which has been extensively used during recent years has become a wide spread problem (4, 6 Although secondary failure is often difficult to evaluate and can in many cases be related to intervening infections irregularities of diet, insufficient dosage of the oral drug or other similar reasons there would nevertheless in many instances of failure with one oral agent seem to be a need for a new and more potent oral drug It is possible that secondary failures might occur also with prolonged use of acetohexamide although such cases have so far not been reported (1, 3 14) The results of the present investigation do not permit of any conclusions in this respect since the diabetics included in the study received acetohexamide only during relatively short periods of time and only primary results of the treatment with this drug can therefore be evaluated

No appreciable change was observed during the course of the present study in the diabetic control of the group of patients in whom previous treatment with carbutamide was replaced by

hexamide were compared with the corresponding values obtained during treatment with the other oral agents, acetohexamide was found to be significantly more effective than tolbutamide both when used alone or in combination with DBI No appreciable difference in the degree of diabetic control could be noted in the group of patients who had previously received carbutamide whilst the average excretion of glucose increased following replacement of chlorpropamide by acetohexamide

The usefulness of acetohexamide in the treatment of maturity onset diabetes is discussed on the basis of previous reports and the findings of the present investigation and it is concluded that this drug can be expected to be effective in the first place in such diabetic patients in whom secondary failure has occurred following treatment with tolbutamide although occasional chlorpropamide failures can probably also benefit from treatment with acetohexamide

Subjective signs of intolerance during the treatment were remarkably few and hypoglycemic symptoms were encountered in only one case The effects of acetohexamide on liver function and hematopoiesis were not investigated during the course of the present study

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drugs was found to be more effective than the combined treatment with tolbutamide and equivalent amounts of DBI. The combined use of two oral antidiabetic drugs which exhibit different pharmacological properties seems to offer certain advantages and our previous experience (10, 11) as well as that of others (2, 22) seems to indicate that in a substantial group of maturity onset diabetics who cannot be controlled with a single oral agent diabetic control can be maintained in this way without resorting to insulin treatment. The use of acetohexamide in combination with DBI would seem to offer an additional possibility for combined therapy which can be useful especially in secondary failures with the use of single sulfonylurea drugs.

Acetohexamide was on the other hand found to be on an average less effective than chlorpropamide in the patients who at the same time received DBI although a few individual cases seemed to do better during the period of treatment with acetohexamide. Similar observations have been made by Szanto (21) who used acetohexamide in combination with another biguanide derivative. The fact that improvement of diabetic control was observed in some cases following replacement of chlorpropamide with another on an average less potent hypoglycemic oral agent is interesting and would seem to imply that the insulin reserve of the pancreatic islands was not completely exhausted in these patients. It is thus possible that these cases represent secondary failures to chlorpropamide, which reacted favourably to acetohexamide therapy.

It can be concluded that acetohexamide seems to be a relatively potent sulfonylurea derivative, which is often more effective than tolbutamide. As far as can be determined on the basis of the present investigation and previous reports very few side effects are encountered with its use. Although the results of the present short time study seem favourable further trials with this drug are warranted and an evaluation of its ultimate place in the treatment of diabetic patients can be made only after further adequately controlled studies, which will permit of conclusions regarding the long term effects of the new antidiabetic oral agent, have been published.

Summary

Acetohexamide was administered to 54 previously treated maturity onset diabetic patients. The previous treatment consisted in 18 cases of tolbutamide, 10 patients received carbutamide and 10 were treated with chlorpropamide. In 16 additional patients phenylethylbiguanide (DBI) was used as an adjunct to the sulfonylurea drugs as well during the previous treatment as during acetohexamide therapy. The previously used sulfonylurea compounds were replaced by equivalent amounts of acetohexamide during one or several short periods of treatment after which the patients were each time switched back to the previously used oral drug. The average length of the combined periods of treatment with acetohexamide was 2.5 months.

When the average values for blood glucose and urinary glucose excretion obtained during treatment with aceto

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Plasma Lipids, Including Individual Phospholipids, in Pregnant Rats

By

Olle Vikrot

During human pregnancy most plasma lipids increase considerably (1 4, 20) In animal pregnancy various findings have been observed In some species, e g rabbit (7, 15) and cow (5) a pronounced lipopenia occurs but in other species e g the rat (6 11 13 17 18) hyperlipemia is found The most characteristic finding in the hyperlipemia of human pregnancy is an alteration of the phospholipid pattern in plasma with a relative as well as absolute decrease of lysolecithin (20 21) It was considered of interest to see whether similar changes occur in animals The purpose of the present study was to investigate whether similar alterations in plasma lipids occur in the pregnant rat as during human pregnancy

Material and methods

Ten female rats of the Sprague Dawley strain were used They were 3—6 months old and weighed about 250 grams Initially about 2 ml blood was taken from the tail vein from each of the rats Then six of the rats were mated while four were kept as controls

During the end of pregnancy (1—3 days before delivery) blood was again taken from the pregnant rats and at the same time (3—6 weeks after the initial sample) samples were also obtained from the controls About three weeks after delivery blood was once again drawn from the rats in the pregnancy group During the experimental period the diet was the same in both groups (rat pellets manufactured by Anticimex Stockholm Sweden) and no food restriction was imposed before blood sampling

Blood was collected in heparinized tubes immediately chilled and centrifuged in the cold Usually 1 ml of plasma could be obtained in a few cases only 0 5 ml Single extractions were then carried out and determinations of total and individual phospholipids cholesterol and triglycerides were made in duplicate from each extract (20 21)

In view of the small groups with unequal variances differences between groups were judged by a non parametric rank test (19 p 117)

Results

On thin layer chromatography extracts from rat plasma showed four phospholipid spots with the same mobility as that

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he co trol g oup n=number of animals. PE phospha dylethanolamine Lec lecithin Sph=

% of to al P l p ds				mM			
PE	Lec	Sph	LL	PE	Lec	Sph	LI
1 6	63 8	13 2	21 3	0 03	1 18	0 24	0 39
0 06	0 61	0 60	0 73	0 002	0 054	0 018	0 022
4 3	81 7	9 1	4 9	0 12	2 26	0 25	0 13
0 43	0 8	0 50	0 60	0 013	0 191	0 019	0 015
2 2	70 5	13 6	13 7	0 05	1 58	0 30	0 30
0 23	1 74	0 95	1 36	0 005	0 132	0 020	0 032
1 3	64 7	13 4	20 6	0 02	1 09	0 23	0 35
0 23	0 41	0 25	0 70	0 004	0 021	0 005	0 010
1 4	68 1	13 2	17 3	0 03	1 38	0 27	0 35
0 28	0 87	1 19	0 82	0 006	0 065	0 022	0 022

corr spond ng chang s n he control group P refers to the s gnificance of he differences be ween

of o al P l p ds				mM			
PE	Lec	Sph	LL	PE	Lec	Sph	I L
2 7	17 9	4 1	16 4	0 09	1 08	+0 00	0 26
0 43	1 25	0 94	1 02	0 012	0 193	0 026	0 02
0 1	3 4	0 2	3 3	0 01	0 29	0 04	0 00
0 43	0 5	1 06	0 71	0 009	0 047	0 021	0 018
0 01	0 01	0 0	0 01	0 01	0 01	N.S	<0 01

n t al to the pregnant sample and correspond ng changes n tl e control group

Tl ere was a con s ent and s gn f cant cl ange n the pl osphol p d pattern dur g pregnancy tl a pronounced al olu e and ela e decreas n l solec l n Tl e absolu e alues for spl ngomyel n d d not change but there v as a sl gl t de rease in percentage value Lecith n and phosphat dyletl anolam ne increased both absolutely and relati ely Tl e total amount of phospl ol p ds n creased but ot s gn ficantly more than i the control group The increase n cl oles erol v as also not s gn ficant but

TABLE I Mean plasma lipids and standard errors of the means (S E M) in pregnant rats and in sphingomyelin LI = lysolecithin

Blood sample	Triglycerides mM	Cholesterol mM	Total phospholipids mM
Pregnant group (n = 6)			
Before pregnancy	0 28	2 09	1 85
S E M	0 054	0 101	0 087
During pregnancy	3 46	2 59	2 75
S E M	0 671	0 183	0 209
In the puerperium	0 38	2 28	2 23
S E M	0 066	0 143	0 153
Control group (n = 4)			
Initial sample	0 18	1 90	1 68
S E M	0 028	0 059	0 024
Repeat sample	0 38	2 10	2 03
S E M	0 132	0 149	0 083

TABLE II Mean changes from initial to pregnant values of plasma lipids in the pregnant group and the two groups N S = not significant Other symbols as in table I

	n	Triglycerides mM	Cholesterol mM	Total phospholipids mM
Pregnant rats	6	+3 19	+0 50	+0 91
S E M		0 660	0 237	0 215
Control rats	4	+0 20	+0 20	+0 35
S E M		0 154	0 190	0 064
P		<0 01	N S	N S

of the four main spots obtained from human plasma Spray tests with ninhydrin and Dragendorff reagent showed the same results as on separation of human plasma phospholipids (*21*), and thus the four spots were considered to represent phosphatidylethanolamine, lecithin, sphingomyelin and lysolecithin

The recovery of phosphorus applied to the plates averaged 89 per cent about the same as in the analyses of human plasma

Mean values for plasma lipids in the two groups at various times are given in table I Table II presents mean changes in the pregnant group from the

erides or phospholipids (3) When acetate was injected in vivo there was no increased incorporation into liver cholesterol during pregnancy (17), but in vitro studies with liver slices showed increased uptake of acetate into 'unsaponifiable substances' (3) The total output and the concentration of cholesterol in the bile were decreased (16)

Summary

Total and individual phospholipids cholesterol and triglycerides in plasma were determined in six rats before during and after pregnancy and in four non pregnant controls

The phospholipid composition in the non pregnant rat showed a much higher lysolecithin percentage than in humans

During pregnancy the levels of plasma lipid changed in a similar way as in humans

Among the phospholipids lecithin and phosphatidylethanolamine increased Lysolecithin decreased both absolutely and relatively There was however no significant change in the absolute level of sphingomyelin Total phospholipids and cholesterol did not show a significant increase in this study but there was a pronounced increase of triglycerides

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there was a considerable increase of triglycerides

The values obtained 3 weeks after pregnancy had returned towards the normal value but there was still a difference in the phospholipid pattern in comparison with the original values

Discussion

The initial values when the animals were not pregnant differed considerably from lipid values in a human material There seem to be differences in lipid values between different rat strains, but, in general, the values obtained in this study for cholesterol, *triglycerides and total* phospholipids were comparable with those given in the literature

The composition of rat plasma phospholipids has been studied by several groups but with somewhat different results (9, 12, 14) There is a general agreement that the percentage of lysolecithin is higher and that of sphingomyelin *lower in rats than in humans* The values for lecithin and phosphatidyl ethanolamine are roughly the same as in *human plasma The findings in this* study did not differ appreciably from the results of Newman et al (14) and Lyman et al (12), but the statement of Howard et al (9) that 35 per cent of the total phospholipids is lysolecithin, was not verified in the present investigation

During pregnancy in rats several authors (6, 11, 13, 17, 18) have observed a hyperlipemia, most notably in the levels of triglycerides and unesterified fatty acids, while the increases in cholesterol and total phospholipids were relatively slight but significant The findings of the present study are not in disagreement with the earlier reports

The phospholipid composition in rat pregnancy has not been studied previously There was a striking similarity to the findings in human pregnancy (20, 21) The decrease of lysolecithin was *even more pronounced, and all other* changes occurred in the same direction as in humans, with the exception that absolute sphingomyelin values were unaltered in rats but increased in humans

Pregnant rats increase their food intake (18), and it was found that a reduction in the food intake influenced the triglyceride levels (10, 18) However, *the concentrations of cholesterol and* total phospholipids were only slightly altered

The mechanism behind the hyperlipemia of pregnancy is not wholly known (20) The similarity of the plasma lipid alterations during pregnancy in the rat to those in humans favors the hypothesis that the etiology is the same *as in humans*, which suggests an approach to experimental studies of the genesis of this hyperlipemia Some *studies on the lipid metabolism in the* liver of pregnant rats have already been reported Though the liver increased in size the relative amount of cholesterol, triglycerides and total phospholipids was unchanged (2, 3 17), nor did the composition of the phospholipid fraction in the liver seem to be notably changed (8) Studied with P^{32} the turnover of lipid phosphorus in the liver of pregnant rats was increased (2) Incorporation of acetate into fatty acids was increased (3, 17) and the newly synthesized acids were incorporated mainly into triglyc

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From the Hospital for Infectious Diseases and the Central Bacteriological Laboratory, Stockholm, Sweden

Infections with Eaton Agent in Pneumonia

By

Göran Sterner, Arne Svedmyr, Gösta Tunevall and Sigvard Wolontis

Primary atypical pneumonia (P.A.P.) has been differentiated from bacterial pneumonia on clinical and therapeutic grounds since the early forties (9, 13, 22, 25, 2[illegible]). In 1944 Eaton et al. (10) reported the isolation of a filtrable agent from patients with P.A.P. Their suggestion that this agent caused cold agglutinin positive P.A.P. was not confirmed until the reports of Liu 1957 (18) and Liu et al. 1959 (19) appeared. Using the fluorescent antibody technique these authors could demonstrate that infection with Eaton agent was associated with primary atypical pneumonia with or without formation of cold agglutinins. It has now been established that this agent is a member of the genus Mycoplasma (3, 4, 7, 20). Infection with Eaton agent occurs in all ages and appear throughout the year (6). All types of acute respiratory illness, particularly diseases in the lower parts of the respiratory tract may be caused by Eaton agent (11, 12, 21). According to reports from U.S.A. (6, 16, 17) as well as Western Europe (1, 2, 8, 12, 14, 15, 20, 21) Eaton agent seems to be one of the most common causes of P.A.P.

In the present investigation we have examined sera from pneumonia cases admitted during 1962 to the Hospital for Infectious Diseases in Stockholm. Tests for the presence of complement fixing (CF) antibodies against Eaton agent and for cold agglutinins were performed.

Material and methods

Only cases with X-ray verified pneumonia were included in this study. During 1962 no systematic sampling of blood from such cases was carried out. However, frozen paired sera from 73 out of 93 cases with pneumonia admitted during 1962 were available for investigation. The first serum was obtained during the first two weeks of the disease in 64 cases, in the remaining 9 cases during the third week. The interval between first and last serum sampling was generally about two weeks, in some cases considerably more and only in 5 cases 6—8 days. Table I shows the distribution by month of pneumonia cases in the hospital and table II the age distribution. The numbers of investigated cases are also given in proportion to the total numbers of pneumonia cases for every month and age

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Primary atypical pneumonia (P A P) has been differentiated from bacterial pneumonia on clinical and therapeutic grounds since the early forties (9, 13, 22, 25 29) In 1944 Eaton et al (10) reported the isolation of a filtrable agent from patients with P A P Their suggestion that this agent caused cold agglutinin positive P A P was not confirmed until the reports of Liu 1957 (18) and Liu et al 1959 (19) appeared Using the fluorescent antibody technique these authors could demonstrate that infection with Eaton agent was associated with primary atypical pneumonia with or without formation of cold agglutinins It has now been established that this agent is a member of the genus Mycoplasma 3 4 7 20) Infection with Eaton agent occurs in all ages and appears throughout the year (6 All types of acute respiratory illness particularly diseases in the lower parts of the respiratory tract may be caused by Eaton agent 6 12 21 According to reports from U S A 6 16 17) as well as Western Europe 1 2 8 12 14 15 20 21) Eaton agent seems to be one of the most common causes of P A P

In the present investigation we have examined sera from pneumonia cases admitted during 1962 to the Hospital for Infectious Diseases in Stockholm Tests for the presence of complement fixing (CF) antibodies against Eaton agent and for cold agglutinins were performed

Material and methods

Only cases with X ray verified pneumonia were included in this study During 1962 no systematic sampling of blood from such cases was carried out However frozen paired sera from 73 out of 399 cases with pneumonia admitted during 1962 were available for investigation The first serum was obtained during the first two weeks of the disease in 64 cases in the remaining 9 cases during the third week The interval between first and last serum sampling was generally about two weeks in some cases considerably more and only in 5 cases 6—8 days. Table I shows the distribution by month of pneumonia cases in the hospital and table II the age distribution. The numbers of investigated cases are also given in proportion to the total numbers of pneumonia cases for every month and age

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TABLE I Monthly distribution of patients with pneumonia admitted to the Hospital for Infectious Diseases, Stockholm, in 1962

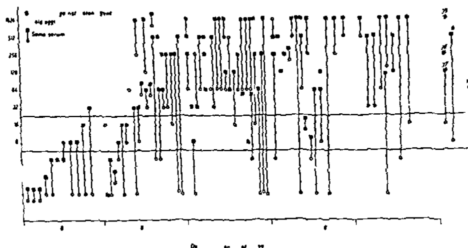
Month	All cases	Investigated cases	CF antibody	
	P.A.P.[1]/Total	P.A.P.[1]/Total	4-fold titre rise	Titre > 256 no rise
I	10/ 56	0/ 0		
II	1/ 25	0/ 1		
III	2/ 34	1/ 5		1
IV	5/ 37	5/ 7	3	
V	6/ 46	2/ 7		1
VI	1/ 27	0/ 2		1
VII	1/ 14	1/ 1		1
VIII	4/ 20	1/ 3	1	
IX	7/ 24	1/ 4	3	
X	17/ 39	8/11	4	5
XI	23/ 41	12/17	10	4
XII	23/ 36	8/15	6	1
Total	100/399	39/73	27	14

[1] Recorded diagnosis

TABLE II Age distribution of patients with pneumonia admitted to the Hospital for Infectious Diseases, Stockholm, in 1962

Age, years	All cases	Investigated cases	CF antibody	
	P.A.P.[1]/Total	P.A.P.[1]/Total	4-fold titre rise	Titre > 256 no rise
– 2	0/ 16	0/ 1		
3 – 6	3/ 12	0/ 2	1	
7 – 15	18/ 30	15/19	10	6
16 – 19	18/ 33	6/ 8	3	2
20 – 29	13/ 32	3/ 5	1	3
30 – 39	13/ 26	2/ 4	2	2
40 – 49	17/ 42	7/15	7	
50 – 59	9/ 55	4/ 7	2	1
60 – 69	4/ 64	1/ 6	1	
70 –	5/ 89	1/ 6		
Total	100/399	39/73	27	14
			41/73	56 %

[1] Recorded diagnosis



F g 1 Ea on agen CF antibody and corresponding cold agglutin n ti es (27 cases w th rise n CF an body 14 cases w h a CF ti re of ≥ 256 bu no rise)

group The nc dences of P A P diagnosis based on cl nical exan nat on and cold agg u n n reaction among n est gated cases and t ose not n est gated a e also presen ed

No a empt was made to sola e Ea on agen n h s s udy of 1962

Fo demons ra on of CF *ant bodies aga nst Ea on ag nt* a m c ome hod developed by I ul on and Dumbell 11 and mod fed by S edmyr et al 28 was used The an gen emplo ed n h s s udy was ob a ned f om Robb ns Labo a ones Inc U S A Ant gen f om he same ba h as used hroughout the s udy f se a f om 1962 The est as always a d ou s n ultaneously on pa ed se a Howe r h s was not the case w h he *o l agglu n n d erm na on* wh ch were per fo med mmed a ly on a n al to he labo a ory a ord ng o a us o ary me hod 24 5 bu w h few ex ep ons on he same se a ha as used fo t e CF tes Spec al p e a ons we e aken o a o d ool ng during an po a on of he blood

To bo t ea ons a fou fold r se n t e was ons de ed as s gn f cant

I o n a es w l d n ons a ed rise n CF e agan La on agent blood was taken agan n Jan ary 1 65 e ? years af er t nfec on Th s h d serum was tes ed for the p esence of CF ant bod es together w h the two sera taken p eviously Thus time the an gen as from ano her ba ch but from the same manufacturer

For ESR maximum values are g en for whi e cell counts the figures found on admis s on

Ches ray films were examined by Dr P O Gribbe M D

Results

As demonstrated n table I most cases w th P A P were admitted to the hospi tal during the last three months of 1962 A majority of those nvestigated (33/39) sho ed serological gns compatible with an Eaton agent nfection accord ng to criteria discussed below

Infections w th Eaton agent were seen n pat ents from 3 to 69 years (table II) A s gn f cant rise of CF antibod es aga nst Eaton agent was demonstrated in 27 pat ents or 37 % of all invest gated pnemon a cases and another 14 patients or 19 % had a h gh t tre (> 256) but

TABLE I Monthly distribution of patients with pneumonia admitted to the Hospital for Infectious Diseases, Stockholm in 1962

Month	All cases	Investigated cases	CF antibody	
	P A P [1]/Total	P A P [1]/Total	4 fold titre rise	Titre > 256 no rise
I	10/ 56	0/ 0		
II	1/ 25	0/ 1		
III	2/ 34	1/ 5		1
IV	5/ 37	5/ 7	3	
V	6/ 46	2/ 7		1
VI	1/ 27	0/ 2		1
VII	1/ 14	1/ 1		1
VIII	4/ 20	1/ 3	1	
IX	7/ 24	1/ 4	3	
X	17/ 39	8/11	4	5
XI	23/ 41	12/17	10	4
XII	23/ 36	8/15	6	1
Total	100/399	39/73	27	14

[1] Recorded diagnosis

TABLE II Age distribution of patients with pneumonia admitted to the Hospital for Infectious Diseases Stockholm in 1962

Age, years	All cases	Investigated cases	CF antibody	
	P A P [1]/Total	P A P [1]/Total	4 fold titre rise	Titre > 256 no rise
– 2	0/ 16	0/ 1		
3– 6	3/ 12	0/ 2	1	
7–15	18/ 30	15/19	10	6
16–19	18/ 33	6/ 8	3	2
20–29	13/ 32	3/ 5	1	3
30–39	13/ 26	2/ 4	2	2
40–49	17/ 42	7/15	7	
50–59	9/ 55	4/ 7	2	1
60–69	4/ 64	1/ 6	1	
70–	5/ 89	1/ 6		
Total	100/399	39/73	27	14
			41/73	56 %

[1] Recorded diagnosis

TABLE IV Recorded diagnosis compared with CF titre against Eaton agent

Titre	No of cases	Bronchopneumonia		P A P	
		Total	Thereof	Total	Thereof
4-fold rise	27	7		20	[1]13
>256 no rise	14	1	[1]1	13	[1]9
<4	19	16		3	[2]1

[1] 4-fold rise in cold agglutinins

[2] 4 fold rise in cold agglutinins and in antipneumolysin pneumococci abundant in sputum

Discussion

The standard method for assay of antibodies against Eaton agent was previously the immunofluorescent technique (14, 18 19) This is a very sensitive method but according to several authors (5, 21), the simple CF test is as specific though not quite as sensitive In our study infection with Eaton agent was demonstrated or at least made very probable in 33 out of 39 cases of P A P (table IV) It might be mentioned furthermore that a more systematic aetiological study performed in the same hospital during the following year disclosed about one fourth of all pneumonic cases to be infected with mycoplasma Apparently such infections were common both in Finland (15) and Sweden (1 2) during these year

Jansson et al (15) demonstrated CF antibodies against Eaton agent in sera taken five months or more after the infection In our investigation 7 out of 9 patients studied had a CF titre > 8 two years after the pneumonia It was not possible however to exclude reinfections with Eaton agent during this interval

As shown in table IV the clinical diagnosis P A P assigned to the records in our series corresponds very well with the serological evidence of a fresh infection with Eaton agent This may depend on the fact that the clinical diagnosis was generally based on a rising titre of cold agglutinins and in this study as well as in previous ones (17 19) a significant rise in cold agglutinin titre or a titre of ≥ 32 without rise was correlated in 80—90 % to proven or probable fresh infection with Eaton agent

On the other hand a serum may have a high titre of CF antibodies against Eaton agent and yet contain no cold agglutinins (fig 1) As a matter of fact infection with Eaton agent (rising CF titre or a titre of ≥ 256) was associated with the development of cold agglutinins (rise or a titre of ≥ 32) in 67 % Nearly the same percentage (68 %) was given by Jansson et al (15) Liu et al (19) and Kingston et al (17) found 58 % and 46 % respectively

As in the Finnish material (15) radiographic changes of the lungs in our cases of proven Eaton agent infection were too variable to allow of any aetiological conclusions to be drawn from these findings alone In the same group of

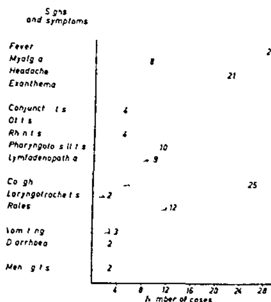


Fig 2 Clinical features in 27 cases with pneumonia associated with Eaton agent infection

TABLE III Complement fixing antibodies against Eaton agent

Highest titre recorded	Number of cases: With 4-fold rise	Number of cases: Without rise
<2	0	0
2	0	4
4	0	15
8	2	10
16	0	3
32	2	0
64	6	0
128	2	0
256	5	4
512	2	3
>1 024	8	7
	27	46
	73	

without rise (table III) Fig 1 demonstrates that high levels of CF antibodies were recorded during the 2nd and 3rd weeks of illness The CF antibodies seem to persist for rather a long time as 7 out of 9 cases still had a titre of > 8 in CF test 2 years after their Eaton agent infection was demonstrated

Fig 1 shows that cold agglutinin occurred in the second week of illness In 67 % of the cases proven or likely to be caused by Eaton agent cold agglutinin showed a significantly rising titre or a titre of ≥ 32 but without rise On the other hand pneumonia cases with a significant titre rise for cold agglutinins or a titre of ≥ 32 without rise were correlated in 93 % to a proven or to probable fresh infections with Eaton agent

Table IV presents the findings of CF antibodies against Eaton agent compared with the diagnosis of the routine record

Some symptoms and clinical findings in the 27 cases of pneumonia with proven Eaton agent infection are given in fig 2 Fever, headache and cough, often severe and paroxysmal, were the dominating symptoms In these 27 cases *X-ray of the chest* showed unilateral changes in 20 cases and bilateral ones in 7 cases One half showed diffuse nodular areas In the other cases confluent infiltrations were often demonstrated sometimes of extensive and massive type with atelectasis No cases with pleurisy were seen, whereas two developed hilar enlargement ESR ranged from between 20 to above 100 mm hour White cell counts varied between 2 000 and 16 000 in most cases being within normal range In 60 % of the cases the neutrophils were slightly increased

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patients we observed many cases with high ESR, even above 100 mm/hour However, we also found the same distribution of ESR in the pneumonia group with no serological evidence of infection with Eaton agent Among our mycoplasma cases none had white cell counts above 17,000 Marked leucocytosis as usual seems to indicate a bacterial infection On the other hand it is well known that bacterial pneumonia may sometimes present a normal white cell count as do most cases of Eaton agent

More characteristic for an infection with Eaton agent seem to be such symptoms as headache and an intensive, paroxysmal cough (fig 2) Furthermore, a patient with Eaton agent pneumonia is seldom seriously ill in spite of having a high and long lasting fever

The aetiological significance of Eaton agent for the appearance of aseptic meningitis in two of our pneumonia cases and some similar ones seen in our hospital have been discussed elsewhere (23)

Eaton agent seems to be rather contagious in closed groups such as military camps (6, 8, 17), homes for children (26) and families (15 16) Family infections were also observed among our cases

Summary

From 73 out of 399 patients with pneumonia admitted during 1962 to the Hospital for infectious diseases, Stockholm, frozen paired sera were available for a serological study of the occurrence of Eaton agent infection Complement fixation (CF) test against Eaton agent was performed as well as determination of cold-agglutinin In 37 % a fourfold rise of CF antibodies against Eaton agent was demonstrated and a further 19 % had a high titre (≥ 256) but without rise These two groups of patients had in 67 % rising cold agglutinin titres or titres of ≥ 32 but without rise On the other hand pneumonia cases with positive cold-agglutination as defined above were in 93 % correlated to a proven or probable Eaton agent infection In some cases retested 2 years later CF antibodies (titre ≥ 8) could still be demonstrated Clinical symptoms and signs in Eaton agent pneumonia are discussed

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From the Medical Service I, Sahlgren s hospital University of Goteborg, Sweden

The Effect of l-hyoscyamine in Tablets with Sustained Release on Gastric Secretion of Acid in Man

By

GERHARD DOTEVALL and ANDERS WALAN

There has been in recent years an increasing interest in the treatment of peptic ulcer with anticholinergic preparations It has been found that with optimal effective doses (14) anti cholinergic drugs inhibit not only basal secretion but also secretion after a standard meal (5 10) as well as secre tion after stimulation by means of histamine and insulin (1 2) Even after maximal histamine stimulation accord ing to Kay (9) inhibition of acid secre tion can be obtained with anticholiner gic drugs (4 12)

The chief disadvantage with atropine and several other anticholinergic drugs is the shortlasting effect on the gastric secretion Even when atropine was given in optimal doses its effect upon the pH of gastric juice did not last longer than 30 minutes

The present study describes a type of anticholinergic administration in tablets with sustained release in which l hyoscyamine the l form of the racem atropine has been embedded in a porous, insoluble, plastic skeleton (Duretter tablets The l hyoscyamine Duretter tab lets were distributed as Egazil from AB Hassle) Tablets of this type have been used with quinidine, diphenylpyraline ephedrine and some other drugs (3, 6 11, 13) Evidence of delayed absorption has been given

The aim of this investigation was to study the effect on gastric secretion of acid with this long acting preparation as compared with that of l hyoscyamine tablets of ordinary type and with that of placebo

Materials and methods

Nine male patients with a recent diagnosis of peptic ulcer which had been proved radiologically were investigated There was no history or sign of present haemorrhage

Of these nine patients seven had a duodenal ulcer and two a gastric ulcer The patients were selected at random They were put on 0 1 mg l hyoscyamine in ordinary tablets 4 times daily or on 0 2 mg l hyoscya mine of the long acting preparation 3 times daily These doses were then progressively increased by 0 1 mg and 0 2 mg respectively

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There has been in recent years an increasing interest in the treatment of peptic ulcer with anticholinergic preparations. It has been found that with optimal effective doses (14) anticholinergic drugs inhibit not only basal secretion but also secretion after a standard meal (5, 10) as well as secretion after stimulation by means of histamine and insulin (1, 2). Even after maximal histamine stimulation according to Kay (9) inhibition of acid secretion can be obtained with anticholinergic drugs (4, 12).

The chief disadvantage with atropine and several other anticholinergic drugs is the shortlasting effect on the gastric secretion. Even when atropine was given in optimal doses its effect upon the pH of gastric juice did not last longer than 40 minutes.

The present study describes a type of anticholinergic administration in tablets with sustained release in which l-hyoscyamine, the l-form of the racemic atropine, has been embedded in a porous insoluble, plastic skeleton (Duretter tablets. The l-hyoscyamine Duretter tablets were distributed as Egazil from AB Hassle). Tablets of this type have been used with quinidine, diphenylpyraline, ephedrine and some other drugs (3, 6, 11, 13). Evidence of delayed absorption has been given.

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Materials and methods

Nine male patients with a recent diagnosis of peptic ulcer which had been proved radiologically were investigated. There was no history or sign of present haemorrhage.

Of these nine patients seven had a duodenal ulcer and two a gastric ulcer. The patients were selected at random. They were put on 0.1 mg l-hyoscyamine in ordinary tablets 4 times daily or on 0.2 mg l-hyoscyamine of the long acting preparation 3 times daily. These doses were then progressively increased by 0.1 mg and 0.2 mg respectively

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TABLE I Gastric secretion of acid in mEq/hr $6^1/_2$—$7^1/_2$ hours (period I), $7^1/_2$—$8^1/_2$ hours (period II) and $8^1/_2$—$9^1/_2$ hours (period III) after administration of optimal, effective doses of l-hyoscyamine in a sustained release tablet, ordinary l-hyoscyamine tablets and after placebo

Subject	Placebo			Ordinary tablet			Sustained release tablet		
	Period			Period			Period		
	I	II	III	I	II	III	I	II	III
1	1.35	0.84	1.00	0.84	1.02	1.74	0.10	0.24	0.58
2	3.42	5.00	3.36	—	—	—	0.06	0.85	1.10
3	4.54	3.43	1.76	2.13	7.32	5.40	1.95	1.67	0.90
4	3.89	4.12	8.58	2.17	3.61	3.39	2.55	2.78	2.76
5	0.43	0.72	1.13	0.46	0.56	0.66	0	0	0.96
6	6.44	6.22	5.13	7.25	5.20	4.80	0.82	0.61	1.75
7	1.85	1.60	2.43	1.16	2.45	1.57	0.52	0.56	0.68
8	6.70	8.60	8.80	—	—	—	3.80	6.80	7.60
9	—	—	—	5.02	3.64	5.71	1.97	2.06	6.04

until in a few days the patients were getting side effects of parasympaticolytic nature. The most usual side effects were dryness of the mouth, blurred vision and constipation. When such symptoms appeared, the dose was reduced to the highest the patient could take without objectionable side effects. Some dryness of the mouth was tolerated, however. The dose arrived at this way was termed the optimal effective dose (OED). The order in which the ordinary tablets and the long-acting tablets were taken varied at random. When OED was reached the first study of secretion was carried out. The average daily dose of ordinary l-hyoscyamine tablets was 1.6 mg and that of the long-acting tablets 2.7 mg. The optimal effective dose was administered 6 hours before the examination (i. e. at 2 a. m.). The patients were examined also after administration of placebo or without treatment. Three examinations were made of six patients and two examinations of three patients.

After a night's fast the patient was examined at 8.00 a. m. A stomach tube of 3 mm internal diameter was passed into the stomach through the nose. The patient was placed on his left side in a semirecumbent position. He was instructed not to swallow the saliva. Continuous drainage of the stomach was carried out at a subatmospheric pressure of 50 mm Hg. Extra manipulation was applied by means of a syringe in order to prevent stagnation of gastric juice in the tube.

After removing the residual secretion from the stomach and establishing that the tube was returning gastric juice satisfactorily, 30-minute collections of basal secretions were made for at least 3 1/2 hours, i. e. 6—9 1/2 hours after administration of the drug.

The pH was determined potentiometrically in a Beckman pH meter and the gastric juice was titrated with n/10 NaOH until pH of 8.0 (total HCl) was reached. The pH of each sample was measured as well as the volume and concentration of total hydrochloric acid. The results are given as mEq/hour total HCl and in pH recorded to the nearest 0.05 unit.

The significance of differences between groups was tested with Student's t test and differences below the 5 per cent level were regarded as significant.

Results

Six patients were examined 3 times each altogether with placebo or without tablets being administered, with ordinary l hyoscyamine tablets being administer ed and with the long acting preparation of l hyoscyamine

The mean value of secretion measured in mEq/30 min and the mean pH of each 30 minute sample are shown in figs 1 and 2 In addition two more patients were each examined with placebo and the long acting prepara tion and one patient with ordinary l hyoscyamine tablets and the long acting preparation

Secretion of HCl after placebo or without treatment compared with secretion after the long acting preparation of l hyoscyamine

As shown in table II the HCl secretion in mEq 60 min was significantly lower than in the control examinations at 6 1 2 – 7 1 2 hours (period I) at 7 1/2— 8 1 2 hours (period II and also 8 1 2 9 1 2 hours (period III) after administration of the long acting prepa ration of l hyoscyamine (p ~ 0 05) Without treatment or after placebo 33 % of 61 samples showed a pH 2 0 With the long acting preparation the pH was 2 0 in 69 % of 62 samples taken 6 9 1 2 hours after administra tion of the dose

Secretion of HCl after placebo or without treatment compared with secretion after ordi nary l hyoscyamine tablets

The results of these examinations are given in table II At period I II and III there was no significant difference compared with the control examinations

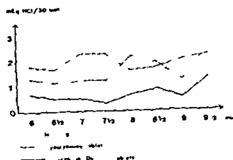


Fig 1 Mean value of gastric secretion of acid per 30 min in 6 patients investigated 6—9 1/2 hours after administration of optimal effective doses of the long acting preparation of l hyoscya mine ordinary l hyoscyamine tablets and placebo or without treatment

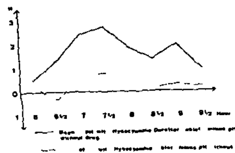


Fig 2 Mean value of pH difference in 6 patients investigated 6—9 1/2 hours after admin istration of optimal effective doses of the long acting preparation of l hyoscyamine ordinary l hyoscyamine tablets and placebo or without treatment

(p > 0 05) Without treatment or after placebo 42 % of 45 samples showed a pH ≥ 2 0 With ordinary l hyoscya mine tablets the pH was ≥ 2 0 in 42 % of 45 samples taken 6—9 1/2 hours after administration of the dose

Secretion of HCl after ordinary l hyoscya mine tablets compared with secretion after the long acting preparation of l hyoscyamine

Comparative investigations of ordinary l hyoscyamine tablets and the long

TABLE II. Mean value of *gastric secretion* of acid in mEq/hr $6^1/_2$—$7^1/_2$ hours (period I), $7^1/_2$—$8^1/_2$ hours (period II) and $8^1/_2$—$9^1/_2$ hours (period III) after administation of the optimal effective doses of l-hyoscyamine *in a sustained release tablet*, ordinary l-hyoscyamine tablets and after placebo

Group comparisons	Period I	II	III
Placebo	3.90	3.82	3.54
Ordinary tablet	2.39	3.36	2.89
% reduction	39	10	13
t-value	1.3	1.1	0.4
significance	>0.05	>0.05	>0.05
Placebo	4.19	4.32	4.02
Sustained release tablet	1.23	1.69	2.04
% reduction	71	61	49
t value	4.0	2.8	2.9
significance	<0.025	<0.025	<0.025
Ordinary tablet	2.76	4.11	3.30
Sustained release tablet	1.13	1.26	1.95
% reduction	59	69	41
t value	1.8	3.2	2.0
significance	>0.05	<0.025	~0.05

acting preparation of l-hyoscyamine were made in 7 cases (table II). The secretion after the long acting preparation was lower in all three periods but a significant difference was found only in period II (i.e. 7 1/2—8 1/2 hours after administration of the dose). With the long acting preparation the pH was > 2.0 in 69 % of 54 samples taken 6—9 1/2 hours after the last dose was given. With ordinary l-hyoscyamine tablets the corresponding figure was 39 % of 53 samples.

Discussion

The anticholinergic drugs most often used during recent years are synthetic preparations of quaternary ammonium type (8). These preparations are as a rule characterized by prolonged effect on account of slow absorption and most often by incapacity to penetrate the blood-brain barrier (7, 8). By means of several of these preparations the acid secretion can be kept low during the greater part of the day. It has been maintained that some synthetic anticholinergics have a more selective effect than atropine. As far as gastric secretion is concerned, however, no conclusive evidence is found of selective effect by the synthetic preparations as compared with atropine. The chief advantages of the synthetic preparations lie in their longer duration, which is probably a consequence of delayed absorption from the gastrointestinal tract. This

makes it possible to obtain a relatively constant tissue concentration over a long period As blood and tissue concentration is proportional to the rate of absorption and excretion (15) the effect of a preparation and degree of side effects due to overdosage must depend on these factors As atropine (and its most active constituent l hyoscyamine) is rapidly absorbed and excreted, the preparation must be administered at short intervals in order to attain a therapeutic level without appearance of side effects l hyoscyamine ought therefore to be suitable for giving in a tablet with sustained release

In a previous investigation (4) we have shown that l hyoscyamine in the long acting preparation was capable of reducing the basal secretion of acid with 72 % 2—3 hours after the last dose compared to 51 % and 60 % after poldine and glycopyrrolate respectively With all preparations the OED had been given The secretion after maximal histamine stimulation was reduced by 40 %, 38 % and 46 % 3—4 hours after the doses of l hyoscyamine poldine and glycopyrrolate respectively

Because of these findings the present study commenced 6 hours after the last dose was given In period 1 6 1/2—7 1/2 hours after the last dose) the reduction of secretion was 71 % which is roughly the same level as that obtained 2 hours after the oral administration of poldine glycopyrrolate and l hyoscyamine in the long acting preparation In the last mentioned investigation 4 the secretion decreased from 4 3 mEq hour before treatment to 1 2 mEq hour during treatment with l hyoscyamine in the long acting preparation — thus to the same level as in the present study where patients, who secreted 4 2 mEq/hour 6 1/2—7 1/2 hours after placebo had a secretion of 1 2 mEq/hour 6 1/2—7 1/2 hours after administration of the optimal dose of the long acting preparation of l hyoscyamine

The reduction of secretion was rather less pronounced 7 1/2—8 1/2 hours after the last dose, and even lower during the last hour of the examination Yet there was still a significant difference as compared with placebo An appropriate interval between doses would therefore appear to be 7—9 hours Wider spacing of the doses would necessitate larger doses with consequently more severe side reactions than with smaller and more frequent doses

The present investigation shows that the slow release preparation of l hyoscyamine may be given in considerably larger daily doses than ordinary l hyoscyamine tablets (on the average 2 7 mg against 1 6 mg a day) without appearance of side effects probably owing to a more even blood and tissue concentration being obtained

Comparative investigations in which secretion has been studied more than 4 hours after an optimal dose of anticholinergic drugs have been rare in the literature Seidelin (12) examined basal secretion 11 hours after an optimal dose of poldine and found that the secretion was 23 % lower than before treatment The difference was not statistically significant however With prolonged anticholinergic treatment Douthwaite and Hunt (5) noted in a few patients a continuation of low secretion for several days after treatment was stopped

TABLE II Mean value of gastric secretion of acid in mEq/hr $6^1/_2$—$7^1/_2$ hours (period I) $7^1/_2$—$8^1/_2$ hours (period II) and $8^1/_2$—$9^1/_2$ hours (period III) after administation of the optimal effective doses of l hyoscyamine in a sustained release tablet, ordinary l hyoscyamine tablets and after placebo

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From the Department of Medicine (Head G Biorck M D) Karolinska Institutet at Serafimerlasarettet, and the Chemical Research Department (Head E Haeffner L E) AB Atomenergi, Stockholm Sweden

Trace Elements in Human Myocardial Infarction Determined by Neutron Activation Analysis

By

P O Wester

The biochemistry of myocardial infarction has attracted increasing interest in recent years Attention has been focused on the possibility of achieving better understanding of certain of its clinical manifestations such as pain, arrhythmia and heart failure by studies of biochemical events From the biochemical point of view myocardial infarction has been and still is, a wide field of investigation The role of the bulk elements especially K has been studied extensively from both the metabolic and the aetiological aspects

Recently the possibility that trace elements may constitute an aetiological factor in coronary heart disease has become evident The reports of a negative correlation between the degree of hardness of drinking water and the mortality from coronary heart disease or other degenerative heart diseases may support this theory 7 32 48) Moreover certain trace elements influence enzymic reactions in fat metabolism Chromium and manganese have been shown to stimulate the synthesis of cholesterol whereas vanadium has been reported to diminish it (10) Administration of CaNa EDTA has been stated to reduce the serum cholesterol and to increase the urinary output of certain trace elements, especially Zn (34)

Trace elements usually exert their action in enzymic reactions Some enzymic changes in the serum of patients with myocardial infarction are well documented and are used diagnostically in clinical practice (6, 15, 27, 28) Enzymic alterations have also been shown to occur in the infarcted heart tissue (2 6 39 54) Some reports of changes in the concentration of certain trace elements in the serum of patients with myocardial infarction have also appeared Thus Vallee (52) and Adelstein et al (1) found an increase in serum Cu Hanson and Biorck (18) an increase in serum Cu and a decrease in serum Fe Kanabrocki et al (24) an increase in serum Mn Wacker et al (55 56) a decrease in serum Zn and D Alonzo et al (11, 12) an increase in serum Ni Mo and B In addition in

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Summary

A comparative investigation of the effect on basal secretion of acid in patients with peptic ulcer disease was carried out, using l-hyoscyamine in ordinary tablet form, in a form of preparation allowing sustained release (Duretter tablets) and also placebo. The basal secretion was followed from 6 1/2 to 9 1/2 hours after administration of the dose. With the long-acting preparation of l-hyoscyamine a significant reduction in secretion was shown during the whole of this *period*. The decrease after 6 1/2—7 1/2 hours, 7 1/2—8 1/2 hours and 8 1/2—9 1/2 hours was 71 %, 61 % and 49 % respectively as compared with the control studies. After ordinary l hyoscyamine tablets no significant differences as compared with the control studies were obtained.

The sustained release tablets of l-hyoscyamine may be given in considerably larger daily doses than ordinary l hyoscyamine tablets, without appearance of side effects.

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P O Wester

The biochemistry of myocardial infarction has attracted increasing interest in recent years Attention has been focused on the possibility of achieving better understanding of certain of its clinical manifestations such as pain arrhythmia and heart failure by studies of biochemical events From the biochemical point of view, myocardial infarction has been and still is a wide field of investigation The role of the bulk elements, especially K has been studied extensively from both the metabolic and the aetiological aspects

Recently the possibility that trace elements may constitute an aetiological factor in coronary heart disease has become evident The reports of a negative correlation between the degree of hardness of drinking water and the mortality from coronary heart disease or other degenerative heart diseases may *support* this theory 7 32 48) Moreover certain trace elements influence enzymic reactions in fat metabolism Chromium and manganese have been shown to stimulate the synthesis of cholesterol whereas vanadium has been reported to diminish it (10) Administration of CaNa, EDTA has been stated to reduce the serum cholesterol and to increase the urinary output of *certain trace elements* especially Zn (34)

Trace elements usually exert their action in enzymic reactions Some enzymic changes in the serum of patients with myocardial infarction are well documented, and are used diagnostically in clinical practice (6 15, 27 28) Enzymic alterations have also been shown to occur in the infarcted heart tissue (2 6 39 54) Some reports of changes in the concentration of certain trace elements in the serum of patients with myocardial infarction have also appeared Thus Vallee (52) and Adelstein et al (1) found an increase in serum Cu Hanson and Biorck (18) an increase in serum Cu and a decrease in serum Fe Kanabrocki et al (24) an increase in serum Mn Wacker et al (55 56) a decrease in serum Zn, and D Alonzo et al (11 12) *an increase in* serum Ni Mo and B In addition in

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TABLE I Periodic table The elements determined in the present investigation are in bold type in the

Period	IA	IIA	IIIA	IVA	VA	VIA	VIIA	VIIIA
1	1 H							
2	3 Li	4 Be						
3	11 Na	12 Mg						
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru
6	55 Cs	56 Ba	57—71 La—Lu	72 Hf	73 Ta	74 W	75 Re	76 Os
7	87 Fr	88 Ra		89—102 Actinides				
Rare earths			57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm
Actinides			89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu

vestigations of certain bulk elements in infarcted human heart tissue have been described Thus, a decrease has been shown in K (13, 20, 31), Mg (30) and P (20) and an increase in Na (13, 20, 31) in infarcted tissue as compared with uninjured

Our knowledge of the concentration of trace elements in infarcted human hearts is limited Meister and Schuman reported a large increase in Ca in infarcted human myocardium as compared to normal human heart tissue (30) and Griffith reported a low amount of Mn in heart muscle areas of recent infarction and a high amount in older infarcts (16) Apart from these two reports, little or no information on the trace element concentration in infarcted human heart tissue seems to exist in the literature

A recently developed ion exchange technique based on neutron activation analysis, combined with subsequent γ spectrometry, makes it possible to determine simultaneously a large number of trace elements in the same sample (41—44, 58)

The aim of the present study was to investigate the amounts of Ag As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr Cs, Cu Fe, Hg, K, La Mo, Na P Rb Sb Sc Se, Sm Zn and W in injured and uninjured heart tissue from patients who died of myocardial infarction The position of the elements determined in the periodic table is seen in table I

table

		IB	IIB	IIIB	IVB	VB	VIB	VIIB	0
								1 H	2 He
				5 B	6 C	7 N	8 O	9 F	10 Ne
				13 Al	14 Si	15 P	16 S	17 Cl	18 A
27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu	
95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No		

Material and methods

Heart tissue from 12 autopsy cases with myocardial infarction, 8 men and 4 women ranging in age from 58 to 86 years, was investigated. The bodies had been stored at +4 C pending autopsy, and no obvious signs of autolysis were present. About 1.5 g wet weight of injured and adjacent uninjured heart tissue was dissected out with a glass knife. Fat and connective tissue were removed. The tissue was transferred to weighed quartz ampoules with a glass rod. The ampoules with content were weighed, dried and sealed as previously described [57]. They were then ready for irradiation with thermal neutrons in an atomic reactor. Heart tissue adjacent to the specimens was collected for microscopical examination and nitrogen analysis. Nitrogen was determined according to Kjeldahl. The degree of fibrosis in the uninjured heart tissue from the infarcted hearts was estimated microscopically and graded from + to ++++ according to an arbitrary scale (+ slight perivascular fibrosis, ++ moderate perivascular fibrosis, +++ advanced perivascular fibrosis, ++++ advanced perivascular fibrosis and wide spread interstitial fibrosis) as seen in figs 1–4.

The diagnosis and estimation of the age of the myocardial infarctions were based on both clinical and patho-anatomical data. Particular attention was paid to the occurrence of arterial hypertension (*diastolic pressure* ≥ *100 mm Hg*) *and the administration of diuretics and/or digitalis*.

Case reports

CASE 1. Piano repairer, aged 66. Moderate hypertension had existed for 7 years. He had been treated with digitalis and diuretics for

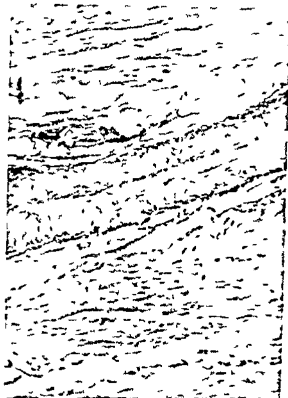


Fig 1 Slight perivascular fibrosis



Fig 2 Moderate perivascular fibrosis



Fig 3 Advanced perivascular fibrosis

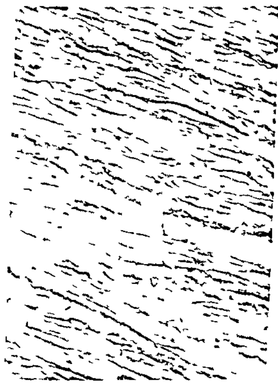


Fig 4 Interstitial fibrosis

the past 3 years owing to heart failure During the last year he had mild angina of effort. Severe retrosternal pain radiating into both arms and not relieved by vasodilator agents, developed suddenly 18 days before hospitalization On admission the ECG showed signs of a relatively recent myocardial infarction SGOT 22 units, SGPT 71 units ESR 67 mm hr WBC 8 600/mm³ Blood sugar 205 mg 100 ml. His temperature was subfebrile On the day he was to be discharged he collapsed and the ECG showed ventricular fibrillation External heart massage and defibrillation were ineffective and he died after one hour's treatment.

Autopsy findings The heart weighed 660 g with hypertrophy of the left ventricle The coronary arteries were highly atherosclerotic with several narrowings, and in places could not be cut A fibrotic scar was visible centrally in the anterior wall of the left ventricle A 6 to 7 week-old myocardial infarction was observed in the posterior wall of the left ventricle There was also a fresh infarction in the superior part of the anterior wall of the left ventricle and in part of the ventricular septum.

Microscopical examination of tissue from the infarcted superior part of the anterior wall of the left ventricle disclosed dissociated structure poorly stainable nuclei partial disappearance of striation and several small haemorrhages.

Specimens were taken from the superior part of the anterior wall of the left ventricle (injured) and from the ventricular septum (uninjured)

CASE 2 Grinder aged 58 Apart from a myocardial infarction 2 years previously he had been healthy Severe chest pain, radiating into both arms, as well as dyspnoea, appeared early in the morning of the day he was hospitalized During the preceding week, some chest pain had occurred On admission he was pale with severe dyspnoea a faint pulse and unmeasurable blood pressure The ECG showed typical signs of a fresh myocardial infarction corresponding to the anterior wall of the left ventricle. He died 1 1/2 hours after admission

Autopsy findings The heart weighed 550 g with hypertrophy of the left ventricle The coronary arteries showed severe atherosclerosis, with several narrowings. Two fibrotic scars were present, one in the ventricular septum and one in the posterior wall of the left ventricle The greater part of the anterior wall of the left ventricle and a thin strip of the ventricular septum were freshly infarcted

Microscopical examination of tissue from the freshly infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei, as well as the presence of a few leukocytes.

Specimens were taken from the anterior (injured and the posterior uninjured wall of the left ventricle

CASE 3 Taxi driver aged 61 Three years previously he had thrombosis of the left internal carotid artery with resulting hemiplegia Acute anxiety and vomiting as well as dyspnoea and pain in the whole body developed suddenly 13 hours before hospitalization On admission he was cyanotic and pulmonary oedema was present The pulse in the radial arteries was impalpable Systolic blood pressure 115 mm Hg The ECG showed suspected signs of a fresh myocardial infarction corresponding to the posterior wall of the left ventricle The patient died 2 hours after admission

Autopsy findings The heart was generally enlarged with moderate hypertrophy of the left ventricle The coronary arteries showed severe atherosclerosis An occluding thrombus was present in the right coronary artery 4—5 cm distal to the aorta. The greater part of the posterior wall of the left ventricle was infarcted A few small fibrotic scars were observed in the border between the ventricular septum and the posterior wall of the left ventricle

Microscopical examination of tissue from the infarcted posterior wall of the left ventricle showed disappearance of striation and of stainable nuclei as well as some leukocytes and numerous small haemorrhages.

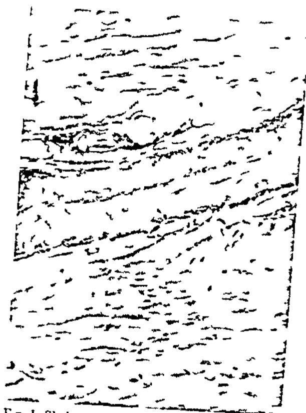


Fig 1 Slight perivascular fibrosis



Fig 2 Moderate perivascular fibrosis



Fig 3 Advanced perivascular fibrosis

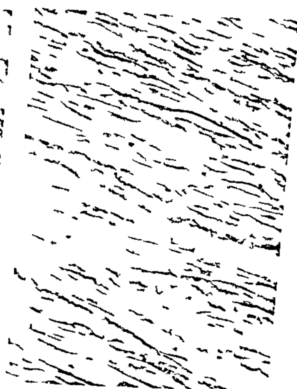


Fig 4 Interstitial fibrosis

the past 3 years owing to heart failure During the last year he had mild angina of effort. Severe retrosternal pain, radiating into both arms and not relieved by vasodilator agents developed suddenly 18 days before hospitalization On admission the ECG showed signs of a relatively recent myocardial infarction SGOT 22 units SGPT 71 units. ESR 67 mm/hr WBC 8 600/mm³ Blood sugar 205 mg/100 ml His temperature was subfebrile On the day he was to be discharged he collapsed and the ECG showed ventricular fibrillation External heart massage and defibrillation were ineffective and he died after one hour s treatment

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Microscopical examination of tissue from the infarcted superior part of the anterior wall of the left ventricle disclosed dissociated structure poorly stainable nuclei partial disappearance of striation and several small haemorrhages

Specimens were taken from the superior part of the anterior wall of the left ventricle (injured) and from the ventricular septum (uninjured)

CASE 2 Grinder aged 58 Apart from a myocardial infarction 2 years previously he had been healthy Severe chest pain radiating into both arms as well as dyspnoea appeared early in the morning of the day he was hospitalized During the preceding week, some chest pain had occurred On admission he was pale with severe dyspnoea a faint pulse and unmeasurable blood pressure The ECG showed typical signs of a fresh myocardial infarction corresponding to the anterior wall of the left ventricle He died 1 1/2 hours after admission

Autopsy findings The heart weighed 550 g, with hypertrophy of the left ventricle The coronary arteries showed severe atherosclerosis with several narrowings Two fibrotic scars were present one in the ventricular septum and one in the posterior wall of the left ventricle The greater part of the anterior wall of the left ventricle and a thin strip of the ventricular septum were freshly infarcted

Microscopical examination of tissue from the freshly infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei, as well as the presence of a few leukocytes

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

CASE 3 Taxi driver aged 61 Three years previously he had thrombosis of the left internal carotid artery with resulting hemiplegia Acute anxiety and vomiting as well as dyspnoea and pain in the whole body developed suddenly 13 hours before hospitalization On admission he was cyanotic and pulmonary oedema was present The pulse in the radial arteries was impalpable Systolic blood pressure 115 mm Hg The ECG showed suspected signs of a fresh myocardial infarction corresponding to the posterior wall of the left ventricle The patient died 2 hours after admission

Autopsy findings The heart was generally enlarged with moderate hypertrophy of the left ventricle The coronary arteries showed severe atherosclerosis An occluding thrombus was present in the right coronary artery 4—5 cm distal to the aorta The greater part of the posterior wall of the left ventricle was infarcted A few small fibrotic scars were observed in the border between the ventricular septum and the posterior wall of the left ventricle

Microscopical examination of tissue from the infarcted posterior wall of the left ventricle showed disappearance of striation and of stainable nuclei as well as some leukocytes and numerous small haemorrhages

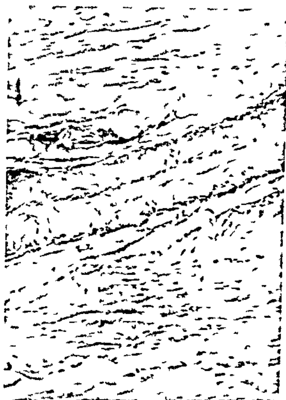


Fig 1 Slight perivascular fibrosis



Fig 2 Moderate perivascular fibrosis

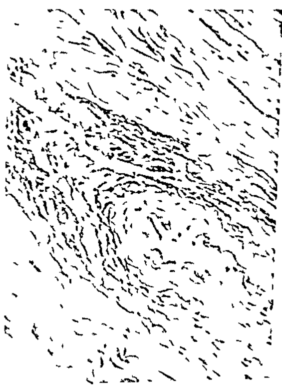


Fig 3 Advanced perivascular fibrosis

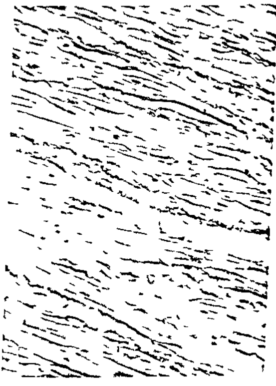


Fig 4 Interstitial fibrosis

Her blood pressure which was 170/110 mm Hg on admission fell to 110/60 mm The ECG showed atrial fibrillation, as well as typical signs of a fresh myocardial infarction She died 4 days later

Autopsy findings The heart weighed 500 g, with hypertrophy of the left ventricle Some coalescences between the aortic cusps were observed but no signs of acute endocarditis The coronary arteries showed severe atherosclerosis. The whole ventricular septum was infarcted.

Microscopical examination of tissue from the infarcted ventricular septum showed disappearance of striation and of stainable nuclei as well as the presence of numerous leukocytes

Specimens were taken from the ventricular septum (injured) and the anterior wall of the left ventricle (uninjured)

CASE 8 Woman aged 74 She had rheumatic fever at age 7 She had suffered from anginal pain for more than 20 years and had a 16-year history of severe hypertension Her first myocardial infarction occurred at age 69 and the second one 4 years later During the past 2 years her condition was aggravated by heart failure She was treated with digitalis and diuretics X ray examination showed a relative heart volume of 700 ml/m² Two days before the last admission severe chest pain appeared it radiated to the left side of her back and left arm and was not relieved by coronary dilators The ECG showed signs of a fresh myocardial infarction corresponding to the anterior wall of the left ventricle SGOT 55 units SGPT 22 units WBC 15 700/mm³ Her temperature ranged from 38 to 39 C She died 3 days after admission

Autopsy findings The heart weighed 570 g with hypertrophy of the left ventricle No signs of valvular heart disease could be detected The coronary arteries were highly atherosclerotic with several severe narrowings especially in the left descending artery A fresh myocardial infarction was present in the anterior wall of the left ventricle and part of the ventricular septum A fibrotic scar was visible in the lower part of the posterior wall of the left ventricle

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei as well as the presence of numerous leukocytes.

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

CASE 9 Former caretaker aged 71 He had no previous symptoms or signs of heart disease On the day before admission, precordial pain appeared radiating into the left arm and the back The ECG on admission showed typical signs of a fresh myocardial infarction corresponding to the anterior wall of the left ventricle The highest value recorded for SGOT was 205 units and for SGPT 70 units ESR 48 mm/hr WBC 14 000 mm³ Blood sugar 248 mg/100 ml His temperature was around 38 C He died suddenly 4 days after admission

Autopsy findings The heart weighed 600 g with generalized hypertrophy The coronary arteries showed severe atherosclerosis A thrombus was present in the left descending coronary artery about 1 cm distal to the bifurcation The whole anterior wall of the left ventricle and an adjacent part of the ventricular septum were infarcted A rupture was visible in the upper part of the infarcted anterior wall The pericardium was filled with blood

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei as well as a moderate number of leukocytes

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

CASE 10 Former tramway conductor aged 78 Diabetes mellitus had been present for 2 years For the past 9 years he had moderate hypertension treated with diuretics and some heart failure fairly well compensated by digitalis In the morning of the day of

Specimens were taken from the posterior wall of the left ventricle (injured) and from the ventricular septum (uninjured)

CASE 4 Pensioner, aged 79 He had no previous symtoms or signs of heart disease Nausea, vomiting and dyspnoea developed 1 1/2 days before hospitalization On admission, he was cyanotic, and the blood pressure was not measurable The ECG showed typical signs of a fresh myocardial infarction, corresponding to the posterior wall of the left ventricle In addition, periodic ventricular fibrillation was recorded He died one day after admission

Autopsy findings The heart weighed 510 g, with hypertrophy of the left ventricle The coronary arteries, particularly the right, were highly atherosclerotic, with several narrowings The whole posterior wall of the left ventricle was infarcted A small fibrotic scar was present in the centre of the wall

Microscopical examination of tissue from the infarcted posterior wall of the left ventricle showed disappearance of striation and of stainable nuclei, and the presence of some leukocytes

Specimens were taken from the posterior (injured) and the anterior (uninjured) wall of the left ventricle

CASE 5 Former housekeeper, aged 86 She was demented, and had been in a home for the aged for the last 11 years Digitalis had been given during the past year, because of some signs of heart failure She was examined by a surgeon, owing to the sudden onset of abdominal pain Her temperature was subfebrile WBC 11,200/mm³ Cholelithiasis was suspected She died suddenly 3 days later

Autopsy findings The heart weighed 350 g, with some hypertrophy of the left ventricle The coronary arteries showed severe atherosclerosis The greater part of the anterior wall of the left ventricle was infarcted A rupture was present in the centre of the infarction The pericardium was filled with blood

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei, as well as a sparse occurrence of leukocytes

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

CASE 6 Woman, aged 58 She had a history of rheumatic fever in youth At age 49, the diagnosis of mitral and aortic stenosis was confirmed by heart catheterization During the past 2 years, her condition had been aggravated by heart failure She was treated with digitalis and diuretics X ray examination showed the relative heart volume to be 710 ml/m² On the day of admission, there was a sudden onset of severe precordial pain, radiating into the left arm The ECG showed signs of a fresh myocardial infarction, corresponding to the anterior wall of the left ventricle Two days later, the SGOT and SGPT were 930 and 720 units, respectively WBC 22,000/mm³ She died after 3 days' hospitalization

Autopsy findings The heart weighed 510 g with hypertrophy and dilatation of both ventricles and atria The mitral and aortic valves were severely stenosed No signs of acute endocarditis were observed The coronary arteries were moderately atherosclerotic, with some narrowings A recent myocardial infarction was present in the lateral part of the anterior wall of the left ventricle

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei as well as a moderate number of leukocytes

Specimens were taken from the posterior (injured) and the anterior (uninjured) wall of the left ventricle

CASE 7 Woman aged 83 She was demented, and had a history of moderate hypertension for 7 years and atrial fibrillation for the past 2 years She had been treated with digitalis and diuretics for the last 18 months Two days after admission to a home for the aged her condition suddenly deteriorated

Her blood pressure, which was 170/110 mm Hg on admission fell to 110/60 mm The ECG showed atrial fibrillation as well as typical signs of a fresh myocardial infarction She died 4 days later

Autopsy findings The heart weighed 500 g with hypertrophy of the left ventricle Some coalescences between the aortic cusps were observed but no signs of acute endocarditis The coronary arteries showed severe atherosclerosis The whole ventricular septum was infarcted

Microscopical examination of tissue from the infarcted ventricular septum showed disappearance of striation and of stainable nuclei as well as the presence of numerous leukocytes

Specimens were taken from the ventricular septum (injured) and the anterior wall of the left ventricle (uninjured)

CASE 8 Woman aged 74 She had rheumatic fever at age 7 She had suffered from anginal pain for more than 20 years and had a 16-year history of severe hypertension Her first myocardial infarction occurred at age 69 and the second one 4 years later During the past 2 years her condition was aggravated by heart failure She was treated with digitalis and diuretics X ray examination showed a relative heart volume of 700 ml m² Two days before the last admission severe chest pain appeared it radiated to the left side of her back and left arm and was not relieved by coronary dilators The ECG showed signs of a fresh myocardial infarction corresponding to the anterior wall of the left ventricle SGOT 55 units SGPT 22 units WBC 15 700 mm³ Her temperature ranged from 38 to 39 C She died 3 days after admission

Autopsy findings The heart weighed 570 g with hypertrophy of the left ventricle No signs of valvular heart disease could be detected The coronary arteries were highly atherosclerotic with several severe narrowings especially in the left descending artery A fresh myocardial infarction was present in the anterior wall of the left ventricle and part of the ventricular septum A fibrotic scar was visible in the lower part of the posterior wall of the left ventricle.

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei as well as the presence of numerous leukocytes.

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle.

CASE 9 Former caretaker aged 71 He had no previous symptoms or signs of heart disease On the day before admission, precordial pain appeared, radiating into the left arm and the back The ECG on admission showed typical signs of a fresh myocardial infarction corresponding to the anterior wall of the left ventricle The highest value recorded for SGOT was 205 units and for SGPT 70 units ESR 48 mm/hr WBC 14 000 mm³ blood sugar 248 mg/100 ml His temperature was around 38 C He died suddenly 4 days after admission

Autopsy findings The heart weighed 600 g with generalized hypertrophy The coronary arteries showed severe atherosclerosis. A thrombus was present in the left descending coronary artery about 1 cm distal to the bifurcation The whole anterior wall of the left ventricle and an adjacent part of the ventricular septum were infarcted A rupture was visible in the upper part of the infarcted anterior wall The pericardium was filled with blood

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei as well as a moderate number of leukocytes

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

CASE 10 Former tramway conductor aged 78 Diabetes mellitus had been present for 2 years For the past 9 years he had moderate hypertension treated with diuretics and some heart failure fairly well compensated by digitalis In the morning of the day of

admission, severe retrosternal pain suddenly developed, it radiated into both arms, especially the left The ECG showed atrial fibrillation and signs of a fresh myocardial infarction, corresponding to the anterior wall of the left ventricle SGOT 300 units, SGPT 120 units His temperature ranged from 38 to 39° C He died 6 days after admission

Autopsy findings The heart weighed 630 g, with hypertrophy of the left ventricle The coronary arteries were highly atherosclerotic, with several narrowings A thrombus was observed in the descending branch of the left coronary artery A myocardial infarction was present in the anterior wall of the left ventricle and adjacent part of the ventricular septum

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei, numerous leukocytes, as well as destruction of muscle cells in patches

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

CASE 11 Civil servant, aged 64 He had no previous symptoms or signs or heart disease Moderate hypertension had been known for 2 years, and was treated with diuretics On the day of admission there was a sudden onset of severe precordial pain, radiating into the left arm The ECG on admission showed suspected signs of a fresh myocardial infarction, corresponding to the anterior wall of the left ventricle SGOT 35 units SGPT 220 units ESR 53 mm/hr WBC 16 600/mm³ His temperature was subfebrile He died suddenly 6 days after admission

Autopsy findings The heart weighed 330 g The coronary arteries showed moderate atherosclerosis A thrombus was observed in the main branch of the left coronary artery A myocardial infarction was present in the anterior wall of the left ventricle The infarcted myocardium had ruptured and the pericardium was filled with blood

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed disappearance of striation and of stainable nuclei, numerous leukocytes, as well as destruction of muscle cells in patches

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

CASE 12 Former clerk, aged 72 He had no previous symptoms or signs of heart disease On the day before admission, there was a sudden onset of severe precordial pain, radiating into the throat and the left shoulder-blade The ECG on admission showed atrial fibrillation and typical signs of a fresh myocardial infarction, corresponding to the anterior wall of the left ventricle SGOT 46 units, SGPT 23 units WBC 12,000/mm³ Blood sugar 98 mg/100 ml His temperature was subfebrile He died 13 days after admission

Autopsy findings The heart weighed 540 g, with hypertrophy of the left ventricle The coronary arteries, especially the left descending artery, were severely atherosclerotic with several narrowings A myocardial infarction was present in the lower part of the anterior wall of the left ventricle and adjacent part of the ventricular septum A parietal thrombus was found on the endocardium corresponding to the infarcted area

Microscopical examination of tissue from the infarcted anterior wall of the left ventricle showed complete disappearance of striation and of stainable nuclei numerous leukocytes as well as advanced destruction of muscle cells

Specimens were taken from the anterior (injured) and the posterior (uninjured) wall of the left ventricle

Neutron activation analysis

The samples were irradiated together with standards in the R 2 reactor at Studsvik with a thermal neutron flux of 2 10^{13} n cm² sec for 24 to 75 hours A decay interval of 2 or 3 days elapsed before chemical processing The ampoules containing the samples were chilled before opening in fluid nitrogen to reduce the pressure induced during irradiation Chemical separation was performed with

TABLE II The bulk elements K Na and P Amounts as µg/g wet tissue

Case no	Age of myocardial infarction	K		Na		P	
		Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
1	2 h			1 320	1 660	1 080	840
2	4 h	2 010	1 990	1 150	1 460	1 100	1 140
3	15 h	2 450	2 470	1 480	1 430	1 640	1 450
4	2.5 d			1 220	1 450	1 230	1 080
5	3 d			1 110	1 510	2 280	1 570
6	3.5 d	2 020	1 680	1 390	1 760	1 470	1 180
7	4 d			1 000	1 710	1 600	1 420
8	5 d			950	1 730	2 290	1 800
9	5 d			1 290	1 840	2 140	1 040
10	6.5 d	2 500	950	1 130	1 890	1 260	550
11	6.5 d	2 170	1 280	1 650	1 700	1 900	1 350
12	14 d			1 450	1 510	2 460	890

TABLE III Trace elements with known biological function Amounts as µg/g wet tissue

Case no	Age of myocardial infarction	Ca		Co		Cu		Fe		Zn	
		Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
1	2 h	35.1	71.9	0.0057	0.0032	1.84	2.11	33.0	23.7	16.8	19.6
2	4 h	29.2	55.9	0.0109	0.0126	2.52	2.54	21.3	40.9	14.4	21.4
3	15 h	63.3	67.1	0.0152	0.0147	2.44	2.39	60.9	44.3	37.3	26.1
4	2.5 d	49.7	84.0	0.0163	0.0151	2.54	2.58	71.3	40.8	27.7	20.3
5	3 d	46.2	105	0.0333	0.0189	2.78	3.58	39.4	62.0	26.1	16.5
6	3.5 d	27.5	110	0.0267	0.0194	2.58	3.05	29.1	66.1	24.3	17.6
7	4 d	66.4	149	0.0121	0.0057	3.52	3.64	20.3	14.7	20.6	12.0
8	5 d	38.6	121	0.0108	0.0075	4.18	1.20	34.0	26.6	29.4	18.5
9	5 d	57.3	151	0.0193	0.0147	2.74	2.96	64.8	46.1	30.2	12.6
10	6.5 d	29.6	115	0.0095	0.00 2	2.63	2.99	23.6	20.7	21.9	10.4
11	6.5 d	27.3	56.6	0.0120	0.0048	3.24	2.16	22.5	25.0	24.1	10.2
12	14 d	64.6	192	0.0087	0.0052	2.96	3.10	28.5	66.5	23.6	11.0

a recently developed ion-exchange technique combined with subsequent γ spectrometry (41—43). In order to separate K-42 from the Na 24 activity a slight modification of the chemical separation system was applied in some determinations (44). The γ spectrometric measurements were carried out with a transistorized 512-channel pulse height analyzer attached to a 3 × 3 NaI(Tl) well type crystal. The elements were identified and quantitatively determined as previously described (57). Chemical recovery corrections were made in accordance with the mean values determined earlier (58).

TABLE IV Trace elements with suspected biological function Amounts as μg/g wet tissue

Case no	Age of myocardial infarction	Ba		Br		Cd	
		Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
1	2 h	0 015	0 016	1 72	1 51	0 006	0 006
2	4 h	0 011	0 012	2 31	1 44	0 010	0 010
3	15 h	0 020	0 023	1 45	1 55	0 014	0 010
4	2 5 d	0 010	0 023	1 02	1 80	0 013	0 020
5	3 d	0 015	0 010	1 90	3 51	0 015	0 017
6	3 5 d	0 003	0 009	1 35	2 43	0 015	0 016
7	4 d	0 027	0 049	1 31	1 35	0 016	0 010
8	5 d	0 019	0 026	1 38	2 50	0 006	0 005
9	5 d	0 027	0 031	1 82	2 36	0 013	0 008
10	6 5 d	0 008	0 032	1 01	1 58	0 005	0 005
11	6 5 d	0 020	0 019	1 23	1 40	0 014	0 013
12	12 d	0 012	0 025	1 85	2 02	0 009	0 018

Statistical methods

The following statistical methods were used in the comparison between normal *heart tissue and uninjured tissue from* infarcted hearts The median values in the two material were compared by Wilcoxon s test (14) The mean values of Zn were compared by Student's *t* test

In the comparison between injured and uninjured tissue from infarcted hearts, the following statistical methods were used The differences obtained were tested by the sign test The dependence of the differences on the age of the myocardial infarction was studied graphically In the case of trace elements where a linear dependence could be discerned, the regression line and its significance were calculated Wilcoxon s test was also used to ascertain whether hypertension or *treatment with diuretics or digitalis had* influenced the trace element concentration in the uninjured heart tissue of patients who died of myocardial infarction In this analysis, Student's *t* test was used for the elements Na, P and Zn

The significances obtained have been expressed as follows

—	not significant	$5\% < p$
*	almost significant	$1\% < p \leq 5\%$
**	significant	$0.1\% < p \leq 1\%$
***	highly significant	$p \leq 0.1\%$

Results

Injured and adjacent uninjured human heart tissue from 12 autopsy cases with myocardial infarction was investigated with respect to the concentrations of Ag, As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, La, Mo, Na, P, Rb, Sb, Sc, Se, Sm, W and Zn K was determined in 5 cases The amounts of the elements are listed in tables II—IV Table II shows the bulk elements K Na and P Table III lists the trace elements with known biological function, table IV the trace elements with suspected biological function and tables V and VI the trace elements without known biological function The concentration is given in *μg/g wet tissue In the unin*

Cr		Mo		Rb		Se	
Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
0 0109	0 0130	0 0276	0 0381	3 06	1 35	0 108	0 149
0 0089	0 0059	0 0307	0 0338	0 80	0 90		
0 0073	0 0051	0 0470	0 0519	3 19	2 50	0 167	0 195
0 0116	0 0278	0 0471	0 0422	0 80	0 65	0 197	0 226
0 0067	0 0031	0 0305	0 0215	2 26	1 86	0 107	0 157
0 0084	0 0078	0 0740	0 0362	1 23	0 98	0 212	0 225
0 0054	0 0132	0 0291	0 0154	2 44	1 33	0 064	0 055
0 0200	0 0322	0 0307	0 0248	2 75	1 89	0 186	0 274
0 0214	0 0321	0 0446	0 0158	5 11	1 42	0 179	0 217
0 0047	0 0031	0 0421	0 0265	1 06	0 44	0 165	0 193
0 0109	0 0057	0 0457	0 0314	3 23	1 78	0 153	0 121
0 0060	0 0115	0 0383	0 0083	4 99	2 38	0 232	0 243

jured tissue the mean dry weight was 20 1 ± 0 3 % of the wet weight The corresponding figure for the injured tissue was 19 4 ± 0 4 % The mean nitrogen content of the uninjured tissue was 37 3 ± 0 8 mg N/g wet tissue, and that of the injured tissue 35 3 ± 0 8 mg N/g wet tissue These figures when converted to express the percentage of protein in wet tissue are 23 ± 0 5 and 22 1 ± 0 5 respectively

The degree of fibrosis in the uninjured heart tissue from the infarcted hearts was as follows + in cases 4 6 10 ++ in cases 2 9 11 12 +++ in cases 3 5 7 and ++++ in cases 1 and 8

A comparison between the concentration (in μg g wet tissue) of the elements in normal human heart tissue from autopsy of 20 victims of traumatic accidents determined in a previous study (57) and the corresponding concentration in uninjured heart tissue from autopsy of the 12 cases with myocardial infarction is seen in table VII No significant difference between these groups was observed with respect to most of the elements Cu and Mo were however, somewhat lower in the group with myocardial infarction, and As and Ce somewhat higher A test was made to ascertain whether the concentration of these four elements was dependent on the grade of fibrosis in the uninjured heart tissue No correlation was obtained between the grade of fibrosis and the concentration of As, Ce or Cu The concentration of Mo seemed, however, to decrease with an increasing grade of fibrosis The regression line was almost significant (fig 10)

A comparison between the concentration of the elements in injured and adjacent uninjured heart tissue from the 12 autopsied cases with myocardial infarction is given in table VIII

TABLE V Some trace elements without known biological function Amounts as µg/g wet tissue

Case no	Age of myocardial infarction	Ag		As		Au
		Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue
1	2 h		0 0008	0 00642	0 00624	0 0000297
2	4 h	0 0015	0 0010			0 0000931
3	15 h	0 0014	0 0025	0 00338	0 00298	0 0000394
4	2 5 d	0 0010	0 0021	0 00352	0 00561	0 0000105
5	3 d	0 0009	0 0009	0 00446	0 00498	0 0000148
6	3 5 d	0 0017	0 0039	0 00968	0 0114	0 0000328
7	4 d	0 0011	0 0012	0 00254	0 00104	0 0000082
8	5 d	0 0010	0 0009	0 0353	0 0546	0 0000380
9	5 d	0 0013	0 0015	0 00932	0 00553	0 0000325
10	6 5 d			0 00166	0 00172	0 0000101
11	6 5 d	0 0033	0 0059	0 00599	0 00562	0 0000796
12	14 d	0 0010	0 0011	0 0153	0 0124	0 0000254

TABLE VI Additional trace elements without known biological function Amounts as µg/g wet tissue

Case no	Age of myocardial infarction	La		Sb	
		Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
1	2 h	0 00014	0 00014	0 0014	0 0036
2	4 h	0 00010	0 00011	0 0004	0 0004
3	15 h	0 00028	0 00018	0 012	0 031
4	2 5 d	0 00008	0 00035	0 0044	0 011
5	3 d	0 00026	0 00038	0 0044	0 0033
6	3 5 d	0 00026	0 00033	0 0037	0 022
7	4 d	0 00012	0 00013	0 0023	0 0033
8	5 d	0 00014	0 00015	0 0014	0 0022
9	5 d	0 00013	0 00018	0 0019	0 0021
10	6 5 d	0 00015	0 00020	0 0025	0 0037
11	6 5 d	0 00056	0 00066	0 022	0 023
12	14 d	0 00037	0 0011	0 0017	0 0019

Columns 3—6 show the median and the range in the uninjured and injured tissue, respectively In column 7 are seen the differences between the concentration of the elements in uninjured and in injured tissue Each difference is expressed as the median of the differences Column 8 lists the degree of significance of the difference, tested by the sign test The signs + and — in the last

	Ce		Cs		Hg	
Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
0 0000306	0 015	0 021	0 0188	0 0071	0 0456	0 0370
0 0000828	0 0038	0 0029	0 0045	0 0048	0 0174	0 0148
0 0000870	0 0047	0 0033	0 0085	0 0073	0 0360	0 0423
0 0000379	0 0039	0 015	0 0046	0 0041	0 0513	0 0486
0 0000116	0 0039	0 0098	0 0076	0 0061	0 0232	0 0222
0 000143	0 0050	0 0055	0 0100	0 0060	0 114	0 120
0 0000106	0 0065	0 013	0 0086	0 0053		0 0238
0 0000314	0 0032	0 0066	0 0133	0 0112	0 0392	0 0909
0 0000282	0 0073	0 014	0 0180	0 0049	0 133	0 540
0 0000554	0 0036	0 0089	0 0049	0 0019	0 0258	0 0279
0 000111	0 0077	0 0081	0 0143	0 0074	0 0678	0 0579
0 0000404	0 0075	0 015	0 0182	0 0081	0 0820	0 0542

Sc		Sm		W	
Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
0 000006	0 000006	0 0010	0 0022	0 0016	0 0041
0 000021	0 000039	0 00085	0 0017	0 0014	0 0006
0 000019	0 000028	0 00072	0 00075	0 0034	0 0022
0 000031	0 00010	0 00014	0 00065	0 0026	0 0038
0 000078	0 000018	0 0011	0 0010	0 0049	0 0052
0 000025	0 000025	0 00073	0 0011	0 014	0 0065
0 000009	0 000012	0 00035	0 00049	0 0013	0 0021
0 000011	0 000012	0 00077	0 0020	0 0007	0 0018
0 000016	0 000013	0 0013	0 0011	0 0011	0 0002
0 000009	0 000006	0 00054	0 00069	0 0046	0 0066
0 000038	0 000018	0 0021	0 0037	0 0021	0 0023
0 000022	0 000015	0 0048	0 0067	0 0009	0 0004

column denote whether or not the difference varies with the age of the myocardial infarction

It is evident from this table that considerable differences were found between injured and uninjured tissue with respect to several elements A large increase in Ca was found in the injured heart tissue compared to the uninjured An increase was also recorded for Ba Br

TABLE V. Some trace elements without known biological function. Amounts as µg/g wet tissue

Case no	Age of myocardial infarction	Ag		As		Au
		Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue	Uninjured tissue
1	2 h		0.0008	0.00642	0.00624	0.0000297
2	4 h	0.0015	0.0010			0.0000931
3	15 h	0.0014	0.0025	0.00338	0.00298	0.0000594
4	2.5 d	0.0010	0.0021	0.00352	0.00561	0.0000105
5	3 d	0.0009	0.0009	0.00446	0.00498	0.0000148
6	3.5 d	0.0017	0.0039	0.00968	0.0114	0.0000328
7	4 d	0.0011	0.0012	0.00254	0.00104	0.0000082
8	5 d	0.0010	0.0009	0.0353	0.0346	0.0000590
9	5 d	0.0013	0.0015	0.00932	0.00553	0.0000525
10	6.5 d			0.00166	0.00172	0.0000101
11	6.5 d	0.0033	0.0059	0.00599	0.00562	0.0000796
12	14 d	0.0010	0.0011	0.0153	0.0124	0.0000254

TABLE VI. Additional trace elements without known biological function. Amounts as µg/g wet tissue

Case no	Age of myocardial infarction	La		Sb	
		Uninjured tissue	Injured tissue	Uninjured tissue	Injured tissue
1	2 h	0.00014	0.00014	0.0014	0.0036
2	4 h	0.00010	0.00011	0.0004	0.0004
3	15 h	0.00028	0.00018	0.012	0.031
4	2.5 d	0.00008	0.00035	0.0044	0.011
5	3 d	*0.00026*	*0.00033*	*0.0044*	*0.0033*
6	3.5 d	0.00026	0.00033	0.0037	0.022
7	4 d	0.00012	0.00013	0.0023	0.0033
8	5 d	0.00014	0.00015	0.0014	0.0022
9	5 d	0.00013	0.00018	0.0019	0.0021
10	6.5 d	0.00015	0.00020	0.0025	0.0037
11	6.5 d	0.00056	0.00066	0.022	0.023
12	14 d	0.00037	0.0011	0.0017	0.0019

Columns 3—6 show the median and the range in the uninjured and injured tissue, respectively. In column 7 are seen the differences between the concentration of the elements in uninjured and in injured tissue. Each difference is expressed as the median of the differences. Column 8 lists the degree of significance of the difference, tested by the sign test. The signs + and — in the last

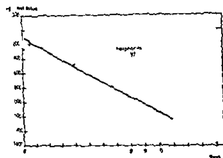


Fig Regression line of the differences in P between injured and uninjured infarcted heart tissue (y) on the age of the myocardial infarction (x)

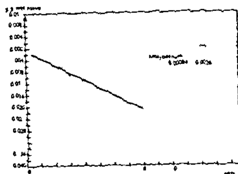


Fig 8 Regression line of the differences in Mo between injured and uninjured infarcted heart tissue (y) on the age of the myocardial infarction (x)

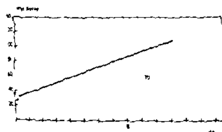


Fig 6 Regression line of the differences in Ca between injured and uninjured infarcted heart tissue (y) on the age of the myocardial infarction (x)

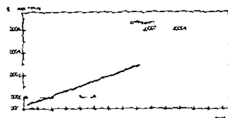


Fig 9 Regression line of the differences in La between injured and uninjured infarcted heart tissue (y) on the age of the myocardial infarction (x)

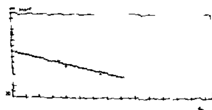


Fig 7 Regression line of the differences in Zn between injured and uninjured infarcted heart tissue (y) on the age of the myocardial infarction (x)

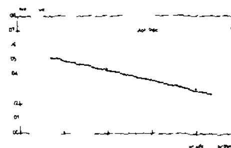


Fig 10 Regression line of the concentration of Mo in the uninjured heart tissue from infarcted hearts (y) on the grade of fibrosis (x)

respectively) and a low amount (but insignificantly decreased) of Fe, Mo and Se ($p \sim 0.07$, 0.07 and 0.06 respectively) in the hypertensive group. In the group treated with diuretics, which coincides with the hypertensive group except for one case, an almost significant decrease in Br (p 0.03) and low amounts (but insignificantly decreased)

TABLE VII Comparison between normal heart tissue and uninjured tissue from infarcted hearts with respect to the concentration of the elements Amounts as µg/g wet tissue

	Element	Normal heart tissue		Uninjured tissue from infarcted hearts		Significance
		Median	Range	Median	Range	
Elements with known biological function	Ca	46	24 – 96	42	27 – 66	–
	Co	0.012	0.0009 – 0.018	0.012	0.0057 – 0.033	–
	Cu	3.8	2.0 – 5.2	2.7	1.8 – 4.2	*
	Fe	35	21 – 53	31	20 – 71	–
	Zn	25	18 – 33	24	14 – 37	–
		Mean 25.5±0.83		Mean 24.7±1.77		
Elements with suspected biological function	Ba	0.020	0.007 – 0.05	0.015	0.003 – 0.03	–
	Br	2.0	1.0 – 4.6	1.4	1.0 – 2.3	–
	Cd	0.012	0.009 – 0.03	0.013	0.005 – 0.02	–
	Cr	0.0062	0.0017 – 0.020	0.0087	0.0047 – 0.021	–
	Mo	0.051	0.026 – 0.13	0.040	0.028 – 0.074	*
	Rb	2.5	1.7 – 5.6	2.6	0.80 – 5.4	–
	Se	0.18	0.097 – 0.25	0.17	0.064 – 0.23	–
Elements without known biological function	Ag	0.0025	0.0006 – 0.025	0.0012	0.0009 – 0.0033	–
	As	0.0024	0.00097 – 0.012	0.0060	0.0017 – 0.035	**
	Au	0.000034	0.0000008 – 0.00011	0.000031	0.0000082 – 0.000093	–
	Ce	0.0016	0.001 – 0.008	0.0049	0.003 – 0.015	*
	Cs	0.011	0.0066 – 0.022	0.0093	0.0045 – 0.019	–
	Hg	0.043	0.000 – 0.096	0.046	0.017 – 0.13	–
	La	0.00029	0.0001 – 0.003	0.00017	0.00008 – 0.0006	–
	Sb	0.0015	0.0006 – 0.004	0.0024	0.0004 – 0.002	–
	Sc	0.000014	0.000003 – 0.0001	0.000020	0.000006 – 0.00004	–
	Sm	0.0025	0.0002 – 0.02	0.00081	0.0001 – 0.005	–
	W	0.0012	0.0007 – 0.002	0.0018	0.0007 – 0.01	–

Na, Sb and the elements belonging to the lanthanide group, Ce, La and Sm, whereas the amount of Co, Cs, P, Rb and Zn was decreased The difference in concentration of certain elements, i e, Ca, La, Mo, P and Zn, increased with increasing age of the myocardial infarction (figs 5—9)

Five of the 12 autopsy cases with myocardial infarction had a history of hypertension, 6 had been treated with diuretics for 1 year or longer, and 6 had been treated with digitalis for 1 year or longer The trace element concentrations in the uninjured heart tissue from *these three groups* was compared to those in the uninjured *tissue from the* autopsy cases which had been normotensive, or had not been treated with diuretics or digitalis for any long period, respectively *A comparison between the hypertensive* and the normotensive *group revealed* an almost significant decrease in Br Co and Se ($p = 0.04$, 0.05 and 0.05,

In ured issue					
M dian	Range		Median of diffe ences	S gnifi cance	Age depend en e
1 680	1 430	—1 890	+3 6	*	
1 160	*549*	1 800	490	**	+
107	6	192	+ 66 2	**	+
0 010	0 0032	0 019	*0 0034*	**	—
2 8	1 2	3 6	0 13		
41	15	67	— 4 3		
17	10	26	10 3		+
0 023	0 009	0 05	*0 005*	*	
1 7	1 4	3 5	+ 0 36	*	—
0 0 0	0 00ɔ	0 02	± 0		
0 0097	0 0031	0 032	0 0008		
0 0 9	0 003	0 052	0 114		+
1 4	0 44	2 5	0 73	*	
0 20	0 0 5	0 27	0 028		
0 0012	0 0008	0 00 9	*0 0002*		
0 00 6	0 0010	0 0	*0 00018*		
0 000048	*0 000010*	0 00011	*0 0000087*		
0 0094	0 003	0 021	+ 0 00ɔ6	*	
0 0061	0 0019	0 013	*0 0034*	**	
0 046	0 01	0 ɔ4	*0 0021*		
0 00018	0 0001	0 001	*0 00005*	*	+
0 003	0 0004	0 03	0 0003	*	
0 000016	0 00006	0 0001	0 0000002		
0 0011	0 000ɔ	0 007	+ 0 0005	*	
0 0023	0 0003	0 007	+ 0 0003		

most trace elements occurr ng n normal l uman heart t ssue had a def n tely ske v distribut on Only the distribut ons of Cu Fe Se and Zn appeared normal

S m lar skew distribut ons were noted n the pre ent study of trace elements in issue from nfarcted human hearts The distribut on of Zn v as the only one — apart from that of the bulk elements Na and P — which appeared normal Con sequently the mean has been gi en only for Na P and Zn v hereas the median has been used as the central value for all the other elements

In reporting the amounts of trace elements n b ological material different

TABLE VIII Comparison between injured and uninjured tissue from infarcted hearts with respect to the concentration of the elements Amounts as μg/g wet tissue

Myocardial infarction					
	Element	Uninjured tissue			
		Median	Range		
Bulk elements	Na	1,250 Mean 1,262±6	950	–	1 650
	P	1,770 Mean 1,729±14	1,080	–	2 460
Trace elements with known biological function	Ca	42	27	–	66
	Co	0 012	0 0057	–	0 033
	Cu	2 7	1 8	–	4 2
	Fe	31	20	–	71
	Zn	24	14	–	37
Trace elements with suspected biological function	Ba	0 015	0 003	–	0 03
	Br	1 4	1 0	–	2 3
	Cd	0 013	0 005	–	0 02
	Cr	0 0087	0 0047	–	0 021
	Mo	0 040	0 028	–	0 074
	Rb	2 6	0 80	–	5 4
	Se	0 17	0 064	–	0 23
Trace elements without known biological function	Ag	0 0012	0 0009	–	0 0033
	As	0 0060	0 0017	–	0 035
	Au	0 000031	0 0000082	–	0 000093
	Ce	0 0049	0 003	–	0 015
	Cs	0 0093	0 0045	–	0 019
	Hg	0 046	0 017	–	0 13
	La	0 00017	0 00008	–	0 0006
	Sb	0 0024	0 0004	–	0 02
	Sc	0 000020	0 000006	–	0 00004
	Sm	0 00081	0 0001	–	0 005
	W	0 0018	0 0007	–	0 01

of Ca and Fe (p = 0 09 and 0 07, respectively) were obtained in comparison with those cases which had not been treated with diuretics for any long period The trace element concentrations in the groups with and without digitalis therapy did not differ significantly

Discussion

Several investigators of trace elements in biological material (19, 35, 51) have observed that most elements are not normally distributed, and the use of the median as central value has been regarded as more accurate than the mean In a previous study (57), I found that

Injured tissue Median	Range			Median of differences	Significance	Age dependence
1 680	1 430	–	1 890	+356	**	–
1 160	549	–	1 800	–490	**	+
107	56	–	192	+ 66 2	**	+
0 010	0 0032	–	0 019	– 0 0034	**	–
2 8	1 2	–	3 6	+ 0 13	–	–
41	15	–	67	– 4 3	–	–
17	10	–	26	– 10 3	*	+
0 023	0 009	–	0 05	+ 0 005	*	–
1 7	1 4	–	3 5	+ 0 36	*	–
0 010	0 005	–	0 02	± 0	–	–
0 0097	0 0031	–	0 032	+ 0 0008	–	–
0 029	0 008	–	0 052	0 114	–	+
1 4	0 44	–	2 5	– 0 73	**	–
0 20	0 055	–	0 27	– 0 028	–	–
0 0012	0 0008	–	0 0059	+ 0 0002	–	–
0 0036	0 0010	–	0 055	– 0 00018	–	–
0 000048	0 000010	–	0 00011	+ 0 0000087	–	–
0 0094	0 003	–	0 021	+ 0 0036	*	–
0 0061	0 0019	–	0 013	– 0 0033	*	–
0 046	0 015	–	0 54	– 0 0021	–	–
0 00018	0 0001	–	0 001	+ 0 00005	*	+
0 0035	0 0004	–	0 03	– 0 0009		–
0 000016	0 00006	–	0 0001	– 0 0000002	–	–
0 0011	0 0005	–	0 007	+ 0 0005	*	–
0 0023	0 0003	–	0 007	+ 0 0003	–	–

most trace elements occurring in normal human heart tissue had a definitely skew distribution Only the distributions of Cu Fe, Se and Zn appeared normal

Similar skew distributions were noted in the present study of trace elements in tissue from infarcted human hearts The distribution of Zn was the only one — apart from that of the bulk elements Na and P — which appeared normal Consequently the mean has been given only for Na P and Zn whereas the median has been used as the central value for all the other elements

In reporting the amounts of trace elements in biological material different

investigators have used different bases of reference, e g wet weight, dry weight ash weight and nitrogen content The use of different bases of reference has made it difficult to compare the results of different investigations In the present study, the concentrations have been expressed on a wet weight basis To facilitate comparison with other investigations, the mean dry weight and the mean nitrogen content are given as well The relation between the three bases of reference given showed good accordance The content of DNA as a basis of reference, although not determined in the present study, might be of some interest

The elements investigated were divided into 4 groups bulk elements, trace elements with known biological function, trace elements with suspected biological function and trace elements without known biological function Obviously, there are no definite borderlines between these groups With increasing knowledge of trace elements in biological material, the group with known biological function will probably increase to the detriment of the other two groups Even classification of an element as a bulk or a trace element may involve certain difficulties Ca in bone and Fe in blood, for example, occur in amounts comparable with the bulk elements However, the amount of these elements in other biological tissues is within the range of the trace elements In the present study, a concentration of 100 p p m was used as the borderline between bulk and trace elements

Some variables, e g age, sex, occupation and geographical location, may influence the concentration of trace elements in tissues The comparison made in table VII between trace element concentration in normal heart tissue and in uninjured tissue from infarcted hearts is based on one autopsy material consisting of 20 victims of accidents (15 males and 5 females aged from 4/12 to 65 years) and on one material consisting of 12 autopsy cases with myocardial infarction (8 males and 4 females aged from 58 to 86 years) The age distribution in these two materials differs considerably, the mean age being 22 8 years in the former and 71 7 years in the latter The sex distribution also differs somewhat (25 % females in the former and 33 % in the latter material) However, no significant differences in the trace element concentration with age or sex could be detected in either material Although a positive correlation between As concentration and age seemed to be present in the normal material, it was not significant ($p = 0\ 2$) This will be discussed in the following

It is known that some trace elements may accumulate in the tissues of certain industrial workers The normal material includes two industrial workers One of them had not been employed longer than a year, but the other had been a cement worker for a long time The heart tissue of this case contained large amounts of As and lanthanides In the present material of cases with myocardial infarction, no industrial worker is represented From a geographical point of view, there are no differences between the two materials All cases came from in or around Stockholm

Most of the trace element concentrations investigated did not differ signifi-

cantly in the two materials The concentrations of As and Ce were, however, higher in the uninjured tissue from infarcted hearts than in the normal heart tissue The difference in As is possibly to be ascribed to the age difference between the two materials In the normal material none of the elements studied varied significantly with age The concentration of As was however somewhat higher in the oldest cases than in the youngest ones This suggests that a significant correlation between As concentration and age might be obtained in a larger normal material Cu and Mo occurred in significantly lower concentration in the uninjured tissue from infarcted hearts than in normal heart tissue

The age of the myocardial infarctions was estimated on the basis of both clinical and patho anatomical data In most cases there was an exact agreement between the clinical and the histological age However in a few cases microscopical estimation gave a slightly higher age than that suggested by the clinical data

It is known that infarctions with a homogeneous appearance on gross examination may exhibit a varying degree of damage when examined microscopically (23 40) This may imply some difficulties when estimating the age histologically Some variation in the degree of damage was in fact observed at microscopical examination of the present material

The comparison between injured and adjacent uninjured tissue from infarcted hearts with respect to the concentrations of the elements (table VIII) discloses significant differences for many elements It seems reasonable to presume that a damaged tissue, such as infarcted myocardium, will release many of its constituents This did, in fact, apply to some of the elements investigated Other elements were on the contrary, found in raised concentration in the injured tissue

In animals with experimental myocardial infarction, a low K concentration in the injured tissue has been reported by several workers (9 21—23, 37 40) In electrolyte steroid cardiopathy, the necrosed heart tissue has been stated to have a low K content (33, 50) Several workers have been able to produce myocardial necrosis in experimental animals by feeding them on a diet low in K and rich in Na (29 36 38 49 50) The injured tissue of infarcted human hearts has also been reported to have a high Na content and low K content (13 31) In the present investigation the Na content of injured cardiac tissue was found to be significantly increased K was determined in only 5 cases In two of them with an infarction less than one day old there was no difference in K concentration between the injured and the uninjured tissue In the other three cases in which the infarction was 3 1/2 6 1 2 and 6 1 2 days old respectively the concentration of K was however markedly decreased in the injured tissue

The third bulk element investigated P has been reported to decrease in myocardial necrosis produced in experimental animals this applies to both acid soluble P 26 and total tissue P (23) In the present investigation the P concentration was found to be significantly lower in injured than in adjacent unin

jured tissue It is seen in fig 5 that this decrease in P was enhanced with increasing age of the myocardial infarction (significance***)

In the group of trace elements with known biological function, Ca was found to be highly enriched in the injured tissue Thus, the median value of Ca in injured tissue was 2 1/2 times that in uninjured tissue Meister and Schumann (30), who investigated the Ca content of infarcted human hearts, also found a large increase in injured tissue but, in addition, some increase in uninjured tissue compared to normal heart tissue On the other hand, Van Peenen and Patel found that Ca was slightly decreased in the heart of patients with arteriosclerotic heart disease (53)

The increase in Ca recorded in infarcted tissue varied with the age of the infarction, as seen in fig 6 (significance**) Some enrichment was seen in the cases with early myocardial infarction, and then a further increase with increasing age of the infarction Using histochemical methods, Yokoyama et al (60) showed that dystrophic calcification occurred in the cytoplasm of the early infarcted myocardial fibres of dog hearts As pointed out by Jennings and Wartman (23), such early calcification is remarkable, in view of the fact that calcification of injured tissue is usually considered to be a late event

Among the other trace elements with known biological function, Co and Zn showed a decrease in injured tissue The reduction of Zn is represented graphically in fig 7 With increasing age of the myocardial infarction, less Zn was present in the injured tissue However, the two cases with the earliest infarctions did, in fact, have an increased amount of Zn in the injured tissue A reduction of Zn in injured tissue is in agreement with the fact that lactic dehydrogenase, a Zn enzyme, disappears from infarcted heart tissue (2, 39) An increase in lactic dehydrogenase activity in the serum of patients with myocardial infarction is well documented, and is used in the diagnosis of this disease (6, 59) The serum Zn concentration, however, has been reported both as elevated (55, 56) and as reduced (11, 12) in patients with myocardial infarction An elevated Cu concentration has been reported in the serum of patients with myocardial infarction (1, 18, 52), and a reduced activity of cytochrome oxidase, a Cu enzyme, has been demonstrated in the infarcted rat and dog heart (2, 39) In the present investigation, however, no significant difference was found between the Cu content of injured and uninjured heart tissue

Histochemically, it has been shown that Fe is deposited in the infarcted myocardium of dogs (60)

On the other hand, a reduced amount of myoglobin has been shown in infarcted human heart tissue compared with unaffected areas from the same hearts (3, 8) Myoglobin has also been detected in serum from patients with myocardial infarction (25) Some Fe enzymes have also been stated to decrease in infarcted heart tissue Thus, the content (4 5, 8) and the activity (39) of cytochrome c and the activity of succinic dehydrogenase (2, 39, 54) have been reported as decreased in infarcted myocardium compared with uninjured Moreover, a def-

inite but inconstant decrease in Fe has been reported in the serum of patients with myocardial infarction (18) In the present investigation, no significant difference in the concentration of Fe was found between injured and uninjured tissue However, as far as Fe is concerned, a possible source of error in the present study is the varying contribution of blood to the specimens examined

Among the elements with suspected biological function, an increased amount of Ba and Br and a reduced amount of Rb were noted in the injured tissue as compared with the uninjured The close physicochemical relation between Cs, K and Rb is well known These three elements were reduced in the injured tissue The concentrations of Cs and Rb ran parallel, with an average decrease of 36 % in the injured tissue In one case (no 2) the concentration of both Cs and Rb was somewhat increased in the infarcted tissue

In the case of Mo, the sign test disclosed no significant difference between injured and uninjured tissue This was because the Mo concentration in injured tissue was increased in three cases (those with an infarct less than 1 day old) and reduced in the others However when the difference between the Mo concentration in injured and uninjured tissue was plotted against the age of the myocardial infarction a linear relation was found as shown in fig 8 There was no significant difference between injured and uninjured tissue in the concentrations of Cd, Cr and Se

Among the trace elements without known biological function, increased amounts of Sb and of the lanthanides Ce La and Sm were found in the injured tissue The La enrichment varied with the age of the myocardial infarction, as seen in fig 9 The reduced Cs content has been discussed above As regards the concentrations of Ag As, Au, Hg, Sc and W, no significant difference was recorded between infarcted and adjacent uninjured tissue

The possibility of a trace metal imbalance in patients with arterial hypertension has been pointed out by Schroeder (45—47) His statement was based partly on a study of the trace elements in human hypertensive urine which he found to have a raised content of some trace elements, e g Cd and Zn In the present investigation, the concentration of certain trace elements in uninjured heart tissue from autopsy cases with myocardial infarction was, in fact found to differ in the cases a history of hyper tension from that in the cases that had been normotensive However the concentrations of Cd and Zn did not differ significantly between these groups although the mean value of Zn was somewhat lower (22 6 ± 2 1 μg/g wet tissue) in the hypertensive group than in the normotensive one (26 2 ± 2 6 μg/g) Almost significantly decreased amounts of Br Co and Sc and low concentrations of Fe Mo and Se were obtained in the hypertensive group All the hypertensive cases and one with a history of heart failure had been treated with diuretics for 1 year or longer A comparison between these two groups — i e the group treated with diuretics for a long period and the untreated group — with respect to the trace element content of the uninjured heart tissue revealed an almost

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significantly decreased amount of Br and low amounts of Ca and Fe in the treated group Two of the 12 *autopsy* cases (nos 6 and 9) had been treated with mercurial diuretics In this connexion, it is interesting to observe the high amounts of Hg in the heart tissue of these cases An accumulation of Hg in tissue of cases treated with mercurial agents has previously been reported by Griffith et al (17)

Summary

By means of neutron activation analysis, injured and adjacent uninjured human heart tissue from 12 autopsy cases with myocardial infarction have been investigated with respect to the concentrations of 23 trace elements The bulk elements K, Na and P were also determined

A recently developed ion-exchange technique, combined with subsequent γ-spectrometry, was used The following trace elements were determined Ag, As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, La, Mo, Rb, Sb, Sc, Se, Sm, W and Zn

In the injured tissue compared with the uninjured, calculations on a wet weight basis showed a decrease in Co, Cs, K, Mo, P, Rb and Zn, and an increase in Br, Ca, Ce, La, Na, Sb and Sm The differences in Ca, La, Mo, P and Zn are dependent on the age of the myocardial infarction, and the regression lines for these elements are given

The concentration of the trace elements in uninjured tissue from infarcted hearts is compared with that in normal heart tissue, determined in a previous study In the uninjured tissue from infarcted hearts a decrease is found in Cu and Mo, and an increase in As and Ce

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Trace Elements in the Conductive Tissue of Beef Heart Determined by Neutron Activation Analysis

By

P O Wester

The anatomy and histology of the conducting system have been thoroughly investigated in both the human heart and the hearts of various animals (e g 1, 8 9 13, 19) Several studies of some chemical components of this structure have also appeared (e g 2, 5 7, 11 12, 14—16 18 21, 23—27) The bulk elements K and Na in conductive tissue have been studied chiefly in electro physiological relations (e g 6) The K and Na content of the conductive tissue of ox hearts has been investigated by Davies et al (4) Information about the concentration of trace elements in the conductive tissue seems to be limited Using spectrographic methods Helander has recently reported interesting semi quantitative values for Al Ba Cu Fe Li and Rb in conductive tissue of beef hearts (10) 31 other trace elements sought could not be detected with the method used Quantitative determinations of trace elements in the conductive tissue seems however to be completely lacking

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A recently developed ion exchange technique based on neutron activation analysis combined with subsequent γ spectrometry, makes it possible to determine simultaneously a large number of trace elements in a very small sample (20, 24)

The aim of the present study was to ascertain the amounts of Ag As, Au Ba, Br Ca, Cd Ce Co Cr, Cs, Cu Fe, Hg K La Mo Na P Rb Sb Sc, Se Sm, W and Zn in the conductive tissue and adjacent muscle tissue of the beef heart

Material and methods

Sample preparation

Four hearts from healthy cattle were obtained a few hours after the animals death The atrioventricular node (AV node) and about 0 3 g wet weight of adjacent atrial muscle the bundle of His and about 0 3 g wet weight of adjacent tissue from the ventricular septum were investigated The heart was opened with a glass knife after which the AV node and the macroscopically visible parts of the bundle were dissected out with

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TALLE III Trace elements w th suspected b ological funct on Amounts as μg g wet tissue

Element	Ba				Br				Cd			
Specimen no	1		3	4	1	2	3	4	1	2	3	4
Ventr cular sep um	0 005	0 006	0 004	0 03	1 6	1 9	1 5	3 4	0 0008	0 002	0 0009	0 001
Bundle of His	0 02	0 2	0 03	0 1	3 0	2 3	3 1	3 8	0 01	0 008	0 003	0 004
Right atr um	0 004	0 02	0 02	0 04	3 5	3 5	3 3	4 1		0 002	0 003	0 002
AV node	0 02	0 03	0 03	0 05	3 9	3 6	3 7	6 4		0 009	0 004	0 009

Element	Cr				Mo				Rb			
Spec men no	1	2	3	4	1	2	3	4	1	2	3	4
Ventr cular sep um	0 0028	0 0018	0 0010	0 015	0 040	0 096	0 040	0 12	2 1	2 2	5 3	4 8
Bundle of His	0 032	0 038	0 012	0 015	0 076	0 042	0 023	0 13	1 3	0 8	2 7	2 7
R ght a rium	0 0025	0 0036	0 0021	0 0084	0 053	0 061	00 0	0 090	1 5	1 7	5 3	3 5
AV node	0 0092	0 0022	0 0094	0 0085	0 14	0 067	0 060	0 14	1 4	1 2	3 9	2 0

E ement	Se			
Spec men no	1	2	3	4
Ventr cular sep um	0 061	0 047	0 14	0 097
Bund e of His	0 051	0 010	0 070	0 073
R ght atrium	0 043	0 032	0 14	0 063
AV node	0 043	0 042	0 13	0 092

ples were ch lled n l qu d n trogen before being opened so as to reduce the pressure duced during irrad at on Chemical separa tion was perform ed w th a re ently developed on exchange techn que comb ned with subsequent γ spectrometry 20 24 The spec rometric measurements ere carried out with a trans torized 512-channel pulse he ght analyzer attached to a 3 3 NaI Tl well type crystal The elem nts were den fied as prev ously described (22 Quant tat e determinat ons were made af er normal zation of the γ spectrograms obta ned In cases of strong act ty the he ght of the photopeaks of the samples was compared to the he ght of the pho o-peaks of the standards In the present stud the samples were small and some of the act vities obtained were faint In these cases the areas of the photopeaks ere calculated and compared to the areas of corresponding photopeaks of the standards Chemical re covery corrections were made in accordance with the mean values determ ned earlier (24)

Statist cal methods

The mean d fferences gi en n table V ere calculated by Student s *t* test The s gnifi cances obtained have been expressed as follo s

—	not significant	5 % < p
	almost s gn ficant	1 % < p < 5 %
	signif cant	0 1 % < p < 1 %
*	highly s gn ficant	p < 0 1 %

TABLE I The bulk elements K, Na and P Amounts as µg/g wet tissue

Element	K				Na				P			
Specimen no	1	2	3	4	1	2	3	4	1	2	3	4
Ventricular septum	2 100	2 140	2,300	2 580	1,050	1,560	1 060	1 220	1 890	2,170	2 650	2 520
Bundle of His	1,220	790	980	1,270	2 030	2 050	1 840	1,680	1,090	700	1 090	1 020
Right atrium	1,380	1,140	1 890	1 360	1 830	2 060	1,760	1 900	1 650	1 510	1 700	1 460
AV node	1,360	1 250	1 480	840	2 040	2 450	2 170	2 330	1 080	690	1 050	890

TABLE II Trace elements with known biological function Amounts as µg/g wet tissue

Element	Ca				Co				Cu			
Specimen no	1	2	3	4	1	2	3	4	1	2	3	4
Ventricular septum	16	55	29	37	0 0056	0 0060	0 0066	0 0088	2 7	3 7	3 2	3 3
Bundle of His	58	110		99	0 0024	0 0015	0 0023	0 0044	0 57	0 52	0 98	1 2
Right atrium		65	66	72	0 0055	0 0020	0 0057	0 0092	1 8	1 5	2 6	2 1
AV node	110	130	100	117	0 0055	0 0020	0 0049	0 0036	1 0	0 13	1 2	1 1

Element	Fe				Zn			
Specimen no	1	2	3	4	1	2	3	4
Ventricular septum	31	44	33	49	12	21	18	18
Bundle of His	11	8 1	9 1	14	5 5	2 6	5 4	6 6
Right atrium	24	26	28	23	9 3	12	14	11
AV node	*14*	*18*	*17*	*19*	*8 0*	*9 8*	*10*	*5 6*

the aid of glass knives and plastic tweezers The common trunk and the right and left bundles were investigated together The samples were transferred to weighed quartz ampoules with a glass rod The filled ampoules were dried by lyophilization until no further loss of weight occurred (about 24 hours) and sealed as previously described (22) The ampoules were then ready for irradiation with thermal neutrons in an atomic reactor Standard samples were prepared as described in an earlier paper (22) Great care was taken to reduce the risk of contamination of the samples The ampoules and glass and plastic instruments used in the sample preparation were thoroughly cleaned by rinsing with 6 N HCl and demineralized water

Neutron activation analysis

The samples and the standards were irradiated together in the *R2* reactor at Studsvik with a thermal neutron flux of 2 10^{13}n cm sec for 24—75 hours A decay interval of one or two days elapsed before chemical analysis The ampoules containing the sam

Au			
1	2	3	4
0.000015	0.000018	0.000003	0.000003
0.00020	0.000036	0.000048	0.00030
0.000054	0.000009	0.000013	0.000035
0.0014	0.000040	0.000038	0.000088

Ce			
1	2	3	4
0.003	0.01	0.002	0.01
0.008	0.01	0.004	
0.006	0.01	0.01	0.01
0.03	0.02	0.01	0.03

La			
1	2	3	4
	0.0003	0.0002	0.0006
	0.0003	0.0003	0.001
0.0003	0.0003	0.0002	0.001
0.0008	0.0006	0.0006	0.004

Sb			
1	2	3	4
0.0007	0.002	0.001	0.001
0.003	0.006	0.004	0.01
0.003	0.002	0.008	0.008
0.003	0.005	0.007	0.01

W			
1	2	3	4
0.001	0.001	0.002	
0.008	0.003	0.005	0.009
	0.003	0.01	0.003
0.01	0.007	0.003	0.01

The bulk elements (table I) showed different patterns of distribution in the comparison made between conductive tissue and adjacent muscle tissue. The conductive tissue was found to be enriched in Na and poorer in K and P. In this tissue most of the trace elements with known biological function were present in low concentration (table II). Ca, however, was found to be high. Among the elements without known biological function (table IV) high concentrations of Ag, Au and somewhat increased concentrations of Sb, Sc and the lanthanides Ce, La and Sm were noted in the conductive tissue, whereas Cs and Hg occurred in low concentration.

Table V shows the mean differences (expressed in µg/g wet tissue) between the ventricular septum and the bundle of His, between the right atrium and the AV node, between the ventricular septum and the right atrium, and between

TABLE IV Trace elements without known biological function Amounts as µg/g wet tissue

Element	Ag				As			
Specimen no	1	2	3	4	1	2	3	4
Ventricular septum	0 0005	0 0002	0 0003	0 003	0 0020	0 0020		*0 0026*
Bundle of His	0 003	0 001	0 002	0 009	*0 0005*	*0 0014*		0 0084
Right atrium	0 0006	0 0008	0 002	*0 002*	*0 0019*		0 0048	0 0015
AV node	0 002	0 002	0 003	0 01	0 0012	0 0008	0 0033	0 0020

Element	Cs				Hg			
Specimen no	1	2	3	4	1	2	3	4
Ventricular septum	0 0076	0 0093	0 022	0 022	0 18	0 23	0 019	0 014
Bundle of His	0 0040	0 0032	0 013	0 0069	0 067	0 035	0 014	0 040
Right atrium	0 0045	*0 0068*	*0 040*	*0 016*	0 092	0 062	0 018	0 016
AV node	0 0043	*0 0044*	*0 021*	0 012	0 082	0 047	0 016	0 014

Element	Sc				Sm			
Specimen no	1	2	3	4	1	2	3	4
Ventricular septum	0 00001	0 00001	0 000009	0 00005	0 0006	0 001	0 0003	0 002
Bundle of His	0 00002	0 00002	0 00002	*0 0002*	0 001	0 001	0 0009	0 005
Right atrium	0 000007	0 000003	*0 00002*	0 00003	0 0004	0 002	0 0005	0 001
AV node	0 00002	0 00001	*0 00004*	*0 0003*	0 002	0 001	*0 0008*	0 004

Results

The atrioventricular node (AV node), the bundle of His and adjacent atrial and ventricular muscle tissue from four cattle hearts (1—4 in tables I—IV) were investigated with respect to the concentration of Ag, As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, K, La, Mo, Na, P, Rb, Sb, Sc, Se, Sm, W and Zn The amounts of the elements are listed in tables I—IV Table I contains the bulk elements Na, K, and P, table II shows the *trace elements* with known biological function, table *III* the trace elements with suspected biological function and table *IV* the trace elements without known biological function The amounts are expressed in µg/g wet tissue The mean dry weight as a percentage of the wet weight was 21 4 ± 1 0% for ventricular muscle tissue, 19 5 ± 1 6 % for the bundle of His, 19 4 ± 2 0 % for atrial muscle tissue and 16 9 ± 1 9 % for the AV node

Au				Ce			
1	2	3	4	1	2	3	4
0 000015	0 000018	0 000003	0 000003	0 003	0 01	0 002	0 01
0 00020	0 000056	0 000048	0 00030	0 008	0 01	0 004	
0 000054	0 000009	0 000013	0 000035	0 006	0 01	0 01	0 01
0 0014	0 000040	0 000038	0 000088	0 03	0 02	0 01	0 05

La				Sb			
1	2	3	4	1	2	3	4
	0 0003	0 0002	0 0006	0 0007	0 002	0 001	0 001
	0 0003	0 0003	0 001	0 003	0 006	0 004	0 01
0 0003	0 0003	0 0002	0 001	0 003	0 002	0 008	0 008
0 0008	0 0006	0 0006	0 004	0 005	0 005	0 007	0 01

W			
1	2	3	4
0 001	0 001	0 002	
0 008	0 005	0 005	0 009
	0 005	0 01	0 005
0 01	0 007	0 005	0 01

The bulk elements (table I) showed different patterns of distribution in the comparison made between conductive tissue and adjacent muscle tissue The conductive tissue was found to be enriched in Na and poorer in K and P In this tissue, most of the trace elements with known biological function were present in low concentration (table II) Ca however, was found to be high Among the elements without known biological function (table IV) high concentrations of Ag, Au and somewhat increased concentrations of Sb Sc and the lanthanides Ce, La and Sm were noted in the conductive tissue, whereas Cs and Hg occurred in low concentration

Table V shows the mean differences (expressed in µg/g wet tissue) between the ventricular septum and the bundle of His between the right atrium and the AV node between the ventricular septum and the right atrium and between

TABLE IV Trace elements without known biological function Amounts as µg/g wet tissue

Element	Ag				As			
Specimen no	1	2	3	4	1	2	3	4
Ventricular septum	0 0005	0 0002	0 0003	0 003	0 0020	0 0020		0 0026
Bundle of His	0 003	0 001	0 002	0 009	0 0005	0 0014		0 0034
Right atrium	0 0006	0 0008	0 002	0 002	0 0019		0 0048	0 0015
AV node	0 002	0 002	0 003	0 01	0 0012	0 0008	0 0033	0 0070

Element	Cs				Hg			
Specimen no	1	2	3	4	1	2	3	4
Ventricular septum	0 0076	0 0093	0 022	0 022	0 18	0 23	0 019	0 044
Bundle of His	0 0040	0 0032	0 013	0 0069	0 067	0 035	0 014	0 040
Right atrium	0 0045	0 0068	0 040	0 016	0 092	0 062	0 018	0 016
AV node	0 0043	0 0044	0 021	0 012	0 082	0 047	0 016	0 014

Element	Sc				Sm			
Specimen no	1	2	3	4	1	2	3	4
Ventricular septum	0 00001	0 00001	0 000009	0 00005	0 0006	0 001	0 0003	0 002
Bundle of His	0 00002	0 00002	0 00002	0 0002	0 001	0 001	0 0009	0 005
Right atrium	0 000009	0 000003	0 00002	0 00003	0 0004	0 002	0 0005	0 001
AV node	0 00002	0 00001	0 00004	0 0003	0 002	0 001	0 0008	0 004

Results

The atrioventricular node (AV node), the bundle of His and adjacent atrial and ventricular muscle tissue from four cattle hearts (1—4 in tables I—IV) were investigated with respect to the concentration of Ag, As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, K, La, Mo, Na, P, Rb, Sb, Sc, Se, Sm, W and Zn The amounts of the elements are listed in tables I—IV Table I contains the bulk elements Na, K, and P, table II shows the trace elements with known biological function, table III the trace elements with suspected biological function and table IV the trace elements without known biological function The amounts are expressed in µg/g wet tissue The mean dry weight as a percentage of the wet weight was 21 4 ± 1 0 % for ventricular muscle tissue, 19 5 ± 1 6 % for the bundle of His, 19 4 ± 2 0 % for atrial muscle tissue and 16 9 ± 1 9 % for the AV node

were made on the grounds of the functional differences existing between these parts of the heart

Some values for the trace element content of the left ventricle (not the septum) of beef hearts have been presented in a previous study (23) The values obtained in the ventricular septum of beef hearts in the present study conform well with those reported earlier Thus, no obvious differences were recorded between different parts of the left ventricle of beef hearts with respect to trace element concentrations

In the following, comparisons are made between the concentration of each element in the ventricular septum, the bundle of His, the right atrium and the AV node

Ag The highest amount of Ag was found in the conductive tissue The AV node contained as much as the bundle of His Somewhat higher Ag values were obtained in the right atrium than in the ventricular septum

As In three of the four specimens investigated, the conductive tissue had a somewhat lower As content than adjacent muscle tissue In the fourth case however, the reverse applied

Au The bundle of His was found to be richest in Au and then, in declining order the AV node, the right atrium and the ventricular septum

Ba A high amount of Ba was noted in the conductive tissue The concentrations of Ba in the AV node and the bundle of His were about the same Nor was any large difference found between the ventricular septum and the right atrium

Br The Br content of the conductive tissue was somewhat larger than that of adjacent muscle tissue The highest Br values were recorded in the AV node Twice as much Br was present in tissue from the right atrium as in tissue from the ventricular septum

Ca The concentration of Ca was about twice as high in the conductive tissue as in adjacent muscle tissue, and somewhat higher in the AV node than in the bundle of His The figures for the right atrium were somewhat larger than those for the ventricular septum Mazel and Holland reported in turtle hearts, but not in frog hearts, twice as high Ca values in the atrium and sinus venosus as in the ventricle (17)

Cd Some enrichment of Cd was observed in the conductive tissue The atrial values were as large as the ventricular values or slightly larger

Ce Ce like the other lanthanides determined was found to occur in the same concentration or a slightly higher one in the conductive tissue compared with adjacent muscle tissue The right atrium contained the same amount of Ce as the ventricular septum or a little more

Co The lowest concentration of Co was obtained in the bundle of His No large differences were present between the ventricular septum the right atrium and the AV node

Cr In some cases a large enrichment of Cr was observed in the conductive tissue In case 2 however the AV node contained less Cr than adjacent atrial muscle and in case 4 the Cr concentration was the same in the conductive tissue as in adjacent muscle tissue

Cs The differences in the concentration of Cs between the conductive tissue and adjacent muscle tissue ran

TABLE V Mean differences expressed as μg/g wet tissue

Element	Cu	Fe	K	Na	P	Zn
Ventricular septum — Bundle of His	2.4±0.3**	29 ±4.0**	1,220±112**	−680±124**	1,330±179**	12 ±2.4*
Right atrium — AV node	1.0±0.1**	8.3±1.6*	210±151	−360± 51**	650± 59**	3.5±0.8*
Ventricular septum — Right atrium	1.2±0.3*	13 ±4.3	840±150*	−670± 59**	730±183*	5.6±1.5*
AV node — Bundle of His	0.2±0.1	6.5±1.5*	170±215	350±132	−50± 28	3.2±1.6

the AV node and the bundle of His with respect to the concentrations of the elements Cu, Fe, K, Na, P and Zn.

The significances of the differences obtained are denoted by asterisks. Significant differences were noted for the majority of the elements in most comparisons. The comparison between the different parts of the conducting system revealed, however, an almost significant difference only for Fe.

Discussion

Most of the trace elements studied in the present work have previously been found to occur in skew distributions in human heart tissue. Only the bulk elements studied and the trace elements Cu, Fe, Se and Zn had normal distributions (22). The present study is based on only a small number of cases and, in view of the presumed skew distributions, statistical calculations have been limited to the elements which were previously observed in normal distributions in human heart tissue. The mean differences, with the relevant degree of significance, between the different kinds of heart samples with respect to the concentrations of Cu, Fe, K, Na, P and Zn are presented in table V. The element Se was omitted, since no significant differences were obtained for this element.

Beef heart tissue was chosen for this study on the following grounds: (a) the simplicity of dissecting out the conductive tissue, (b) the size and availability of beef hearts, and (c) the fact that a previous study of trace element concentrations in subcellular fractions was made in beef heart tissue (23).

It is known that the conducting system is not a homogeneous tissue but is a complex of muscle tissue, connective tissue and some nerve fibres. Several investigators of conductive tissue have tried in various ways to make corrections for the connective tissue content (e.g. 15). In the present study, however, no such corrections have been introduced. The comparisons between the trace element concentrations in conductive tissue and adjacent atrial and ventricular tissue

were made on the grounds of the functional differences existing between these parts of the heart

Some values for the trace element content of the left ventricle (not the septum) of beef hearts have been presented in a previous study (23) The values obtained in the ventricular septum of beef hearts in the present study conform well with those reported earlier Thus, no obvious differences were recorded between different parts of the left ventricle of beef hearts with respect to trace element concentrations

In the following, comparisons are made between the concentration of each element in the ventricular septum, the bundle of His, the right atrium and the AV node

Ag The highest amount of Ag was found in the conductive tissue The AV node contained as much as the bundle of His Somewhat higher Ag values were obtained in the right atrium than in the ventricular septum

As In three of the four specimens investigated, the conductive tissue had a somewhat lower As content than adjacent muscle tissue In the fourth case however the reverse applied

Au The bundle of His was found to be richest in Au and then in declining order the AV node the right atrium and the ventricular septum

Ba A high amount of Ba was noted in the conductive tissue The concentrations of Ba in the AV node and the bundle of His were about the same Nor was any large difference found between the ventricular septum and the right atrium

Br The Br content of the conductive tissue was somewhat larger than that of adjacent muscle tissue The highest Br values were recorded in the AV node Twice as much Br was present in tissue from the right atrium as in tissue from the ventricular septum

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Ce Ce, like the other lanthanides determined, was found to occur in the same concentration or a slightly higher one in the conductive tissue compared with adjacent muscle tissue The right atrium contained the same amount of Ce as the ventricular septum or a little more

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the AV node and the bundle of His with respect to the concentrations of the elements Cu, Fe, K, Na, P and Zn

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Discussion

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It is known that the conducting system is not a homogeneous tissue but is a complex of muscle tissue, connective tissue and some nerve fibres Several investigators of conductive tissue have tried in various ways to make corrections for the connective tissue content (e g 15 In the present study, however no such corrections have been introduced The comparisons between the trace element concentrations in conductive tissue and adjacent atrial and ventricular tissue

ular septum at a higher concentration in the atrium, and highest in the conductive tissue The mean difference between the ventricular septum and the bundle of His was 680 μg/g wet tissue (significance**), and that between the right atrium and the AV node 360 μg g wet tissue (significance**) The mean difference between the ventricular septum and the right atrium was 670 μg/g wet tissue (significance**) No significant difference was present between the AV node and the bundle of His

The differences obtained agree well with those reported by Davies et al in the ox heart (4) Comparable results have also been reported in toad hearts (3) and in frog and turtle hearts (17)

P The concentration of P ran, on the whole, parallel to that of K Thus it was highest in the ventricular septum, lower in the right atrium and lowest in the conductive tissue The difference in P concentration between the ventricular septum and the bundle of His was significant as was the difference between the right atrium and the AV node The difference between the ventricular septum and the right atrium was almost significant (table V)

Rb The low Rb content of the conductive tissue as compared to adjacent muscle tissue ran as pointed out above parallel to that of Cs and K

Sb A somewhat low concentration of Sb was obtained in the ventricular septum as compared to the right atrium and the conductive tissue

Sc The concentration of Sc was found to be slightly higher in the conductive tissue than in adjacent muscle tissue

Se No obvious differences were observed between the Se content of the different kinds of heart samples Slightly lower values could perhaps be discerned in the bundle of His than in the ventricular septum

Sm The Sm values obtained were — like those of the other rare earths determined — *the same* in the conductive tissue as in adjacent muscle tissue, or somewhat higher

W The lowest W concentration was recorded in the ventricular septum Almost the same value was obtained in the AV node as in the bundle of His

Zn The conductive tissue was found to contain an almost significantly lower concentration of Zn than adjacent muscle tissue (table V) The values for the right atrium were lower than those for the ventricular septum (significance*) The Zn concentration in the AV node did not differ significantly from that in the bundle of His

Most of the trace elements of biological significance are known to exert their effect on enzymes or enzyme systems The differences found in the present study between the trace element concentration in the conducting system and in the adjacent common myocardium may therefore reflect metabolic differences The small amount of some trace elements e g Cu and Fe, obtained in the conductive tissue can be compared to the small number of cytoplasmic organelles known to be present in this tissue The mitochondria contains among other enzymes those of Krebs cycle and the electron transport enzymes, some of which are known to be metalloenzymes e g various cytochromes and

parallel to those obtained for K and Rb About 50 % reduction of *Cs* was noted in the bundle of His as compared to adjacent ventricular muscle, and a somewhat smaller difference between the AV node and atrial muscle The Cs content of the AV node was slightly higher than that of the bundle of His In three specimens a lower Cs value was recorded in atrial muscle than in ventricular muscle In the fourth specimen, however, the reverse relation applied

Cu The Cu values obtained were analyzed statistically (table V) In the conductive tissue, the concentration of Cu was found to be largely reduced The values in the bundle of His were only about one-quarter those in the ventricular septum, the mean difference being 2 4 μg/g wet tissue (significance**) The difference in Cu content between the AV node and the atrial muscle was about half the difference between the bundle and the ventricle There was no significant difference in Cu concentration between the AV node and the bundle of His The ventricular septum was found to have the highest concentration of Cu, with a mean difference of 1 2 μg/g wet tissue as compared to the concentration in the right atrium

Fe The highest concentration of Fe was obtained in the ventricular septum and the lowest in the bundle of His The mean difference, 29 μg/g wet tissue, is significant A smaller but almost significant difference was present between the atrial muscle and the AV node The Fe values in atrial muscle were slightly lower than in the ventricular septum This difference is not, however, significant

Hg Somewhat lower Hg values were found in the conductive tissue than in adjacent muscle tissue The lowest Hg concentration was noted in the bundle of His

K The ventricular septum was found to contain the largest amount of K The bundle of His contained half this amount The mean difference was 1,220 μg/g wet tissue (significance**) The concentration of K in the right atrium was smaller than in the ventricular septum (mean difference 840 μg/g wet tissue, significance*) There was no significant difference in K concentration between the right atrium and the AV node, nor between the AV node and the bundle of His

Most of my results accord with those of Davies et al for ox heart (4) They reported the highest value in the ventricle, a lower value in the right atrium and no difference between the right atrium and the AV node In the bundle of His, however, they reported a higher K value than that in the present investigation

My results can also be compared to those of Danielson with respect to the K concentration in toad hearts He found the highest K values in the ventricle, a lower value in the atrium and the lowest value in the sinus venosus (3)

La The values obtained in the conductive tissue were the same as those in adjacent muscle tissue or slightly higher

Mo No obvious differences in Mo concentration were recorded between the ventricular, atrial and conductive tissues

Na Contrary to K, Na was found in the lowest concentration in the ventric-

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succinic dehydrogenase containing Fe, and cytochrome oxidase containing Cu On the other hand, *certain trace* elements were found in high concentration in the conductive tissue This may indicate that some enzymes are present in large amounts in the conducting system Cholinesterase, which is associated with Ca, has been reported to occur in high amounts in the conducting system (18, 26) The amount of Ca obtained in the present study was, in fact, twice as high in the conductive tissue as in adjacent heart tissue A more complete understanding of the physiological significance of trace element concentration in the conducting system must, however, await further investigations on the biochemistry of this tissue

Summary

By means of neutron activation analysis, samples of four beef hearts taken from the bundle of His and adjacent ventricular muscle, the AV node and adjacent atrial muscle have been investigated with respect to the concentrations of 23 trace elements The bulk elements K, Na and P were also determined

A recently developed ion exchange technique, combined with subsequent γ-spectrometry, was used The following trace elements were determined Ag, As, Au, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, La, Mo, Rb, Sb, Sc, Se, Sm, W and Zn

In the conductive tissue compared to adjacent muscle tissue, calculations on a wet weight basis show lower concentrations of Cs, Cu, Fe, K, P, Rb and Zn in the former, and higher concentrations of Ag, Au, Br, Ca and Na The mean differences (µg/g wet tissue), as well as their degree of significance, between the bundle of His and adjacent tissue from the ventricular septum, between the AV node and adjacent atrial muscle, between the ventricular septum and the right atrium, and between the bundle of His and the AV node are given for the elements Cu, Fe, K, Na, P and Zn

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The Diagnostic Significance of the Serum Concentration of Pathological Proteins (M-Components)

By

ROLF BACHMANN

Pathological proteins (nomenclature, see Bull Wld Hlth Org *30* 447, 1964) (M-components) in serum occur in myelomatosis and macroglobulinemia Waldenstrom In recent years however such components have been observed with increasing frequency in patients without signs of myelomatosis or macroglobulinemia Waldenstrom, but with other diseases or apparently healthy (2, 5, 11—16 18—21 23, 24) In these patients the serum concentration of the M components was usually lower than in patients with myelomatosis or macroglobulinemia Waldenstrom This paper is concerned with the relation between the concentration of different types of serum M components and the clinical diagnoses

Material

The material consisted of 554 patients with serum M-components. The cases were collected from 55 Swedish hospitals during the years 1959—1964 and represent a continuation of the series Waldenstrom investigated in 1961 (22) The series consisted of 323 men and 231 women The mean age of the men was 66 years that of the women 68 years The age distribution is given in fig 1

Methods

Electrophoretic and immunologic classification was done according to Bachmann and Laurell (4) The concentration of the M-components was measured by paper electrophoresis by eluting the M component separately and subtracting from the value found the estimated concentration of other serum proteins with the same paper electrophoretic mobility as the M component

Diagnostic classification

From the data in the hospital records the patients were classified according to the diagnoses below The records covered a period of some weeks to five years after the discovery of the M-components at our laboratory Neither the type nor the concentration of the M components was considered in the establishment of the diagnoses

Myelomatosis (*M*) This diagnosis was based on one or more of the following

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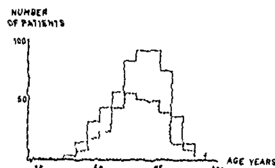


Fig. 1. Age distribution of the material.
[ ] males [ ] females

findings. 1. Skeleton X-ray showed osteolytic lesions for which no other explanation was suspected. 2. Bone marrow smears (a) contained more than 20 % plasma cells (b) contained 10—20 % plasma cells with severe atypia or (c) were considered by the examiner to be typical of myelomatosis but the number of plasma cells was not given. 3. Histological findings at post mortem examination were considered typical of myelomatosis.

Assumed myelomatosis (A). One or more of the following findings gave this diagnosis. 1. Skeleton X-ray showed lesions of presumably osteolytic type. 2. Bone marrow smears (a) contained 10—20 % plasma cells without severe atypia or (b) were found by the examiner to be suggestive of myelomatosis but the number of plasma cells was not given. 3. Histological findings at post mortem examination had allowed a presumptive but not a definite diagnosis of myelomatosis.

Malignant lymphoma (L). To this group were referred patients with macroglobulinemia Waldenstrom, lymphatic leukemia, lymphosarcoma, reticulum cell sarcoma and allied conditions. The diagnosis was based on bone marrow examination, biopsy and/or necropsy. At bone marrow examination an increased number of lymphocytes or lymphocytoid cells gave the diagnosis in patients whose clinical picture did not indicate any other explanation of the findings. No osteolytic lesions should be found on skeleton X-ray.

Non-differentiated neoplasia of reticular tissue (N). The diagnosis was based on histological examination of bone marrow or of biopsy that allowed the diagnosis of neoplasia of reticular tissue but not a more differentiated diagnosis.

Cancer (C). This group included cases of carcinoma, liposarcoma, melanosarcoma, malignant glioma and myeloic leukemia, not assigned to any of the abovementioned diagnostic groups. The diagnoses were in most cases confirmed by histological examination of biopsy and/or necropsy specimens. The cases were divided into 2 subgroups according to whether bone marrow had been examined (C 1) or not (C 2).

Other diseases (O). This group included all other patients and was divided into two subgroups according to whether bone marrow had been examined (O 1) or not (O 2).

Results

Table 1 summarizes the immunologic types of the M-components and their concentration in the various diagnostic groups. In cases with more than one M component the serum concentration given is that of the total of the M components. The concentration of the M components is not given for patients treated with cytostatics 2 weeks or earlier before collection of the samples. The highest concentration of M components of type γG was 10.3 g/100 ml, of type γA 8.6 g/100 ml and of type γM 8.1 g/100 ml. A concentration above 8.0 g/100 ml was found in 13, 3 and 1 cases with M components of type γG, γA and γM respectively.

Immunologic classification demonstrated M-components of type γG in 518 sera, of type γA in 109 sera, of type γM in 95 sera, and of type γ_μ (light-chain proteins) in 18 sera. Seven sera were found to contain both an M-component of type γG and one of type

TABLE I Type and serum concentration of M components in relation to diagnosis Cyt treat = patients who had been treated with cytostatics 2 weeks or earlier before collection of the sample The concentration of the M-components is not given in these cases

M-component		Diagnosis								
Type	Conc g/100ml	M	A	L	N	C 1	C 2	O 1	O 2	Total
G	≤10	8	6	6	–	16	3	53	24	116
	11–20	8	4	2	–	7	1	27	11	60
	21–30	15	10	2	–	–	–	5	–	32
	31–40	13	8	–	1	–	1	1		24
	41–50	14	2	2	–	–	–	2		20
	≥51	44	2	2	–	–	–	2	–	50
	Cyt. treat	12	2	2	–	–	–	–	–	16
	Total	114	34	16	1	23	5	90	35	318
A	≤10	9	4	1	–	7	1	22	4	48
	11–20	11	1	1	–	–	–	–	–	13
	21–30	7	4	–	–	–	–	–	–	11
	31–40	9	–	–	–	–	–	–	–	9
	41–50	6	2	–	–	–	–	–	–	8
	≥51	8	1	–	1	–	–	1	–	11
	Cyt treat	9	–	–	–	–	–	–	–	9
	Total	59	12	2	1	7	1	23	4	109
G–A	≤10	–	–	1	–	2	–			3
	≥11	4	–	–	–	–	–	–	–	4
	Total	4	–	1	–	2	–	–	–	7
M	≤10	–	1	6	1	4	5	10	8	35
	11–20	–	–	11	–	2	–	11	2	26
	21–30	–	1	7	–	–	–	2	2	12
	31–40	–	–	2	1	–	–	–	1	4
	41–50	–	–	6	1	–	–	–	–	7
	≥51	1	–	4	2	–	–	3	–	10
	Cyt treat	–	–	1	–	–	–	–	–	1
	Total	1	2	37	5	6	5	26	13	95
D	≤10	–	–	–	–	–	–	–	1	1
	11–20	1	–	–	–	–	–	–	–	1
	Cyt treat	1	–	–	–	–	–	–	–	1
	Total	2	–	–	–	–	–	–	1	3
	≤10	11	2	–	–	–	–	1	–	14
	11–20	1	–	–	–	–	–	–	–	1
	Cyt. treat	2	–	1	–	–	–	–	–	3
	Total	14	2	1	–	–	–	1	–	18
M p	≤10	–	–	–	–	–	–	3	–	3
	11–20	–	–	–	–	–	–	1	–	1
	Total	–	–	–	–	–	–	4	–	4
	Total	194	48	57	7	38	11	144	53	554

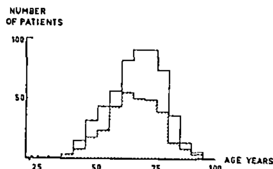


Fig 1 Age distribution of the material
☐ males ☐ females

findings 1 Skeleton X-ray showed osteolytic lesions for which no other explanation was suspected 2 Bone marrow smears (a) contained more than 20 % plasma cells (b) contained 10—20 % plasma cells with severe atypia or (c) were considered by the examiner to be typical of myelomatosis but the number of plasma cells was not given 3 Histological findings at post mortem examination were considered typical of myelomatosis

Assumed myelomatosis (*A*) One or more of the following findings gave this diagnosis 1 Skeleton X ray showed lesions of presumably osteolytic type 2 Bone marrow smears (a) contained 10—20 % plasma cells without severe atypia or (b) were found by the examiner to be suggestive of myelomatosis but the number of plasma cells was not given 3 Histological findings at post mortem examination had allowed a presumptive but not a definite diagnosis of myelomatosis

Malignant lymphoma (*L*) To this group were referred patients with macroglobulinemia Waldenstrom, lymphatic leukemia, lymphosarcoma, reticulum cell sarcoma and allied conditions The diagnosis was based on bone marrow examination, biopsy and/or necropsy At bone marrow examination an increased number of lymphocytes or lymphocytoid cells gave the diagnosis in patients whose clinical picture did not indicate any other explanation of the findings No osteolytic lesions should be found on skeleton X ray

Non differentiated neoplasia of reticular tissue (*N*) The diagnosis was based on histological examination of bone marrow or of biopsy that allowed the diagnosis of neoplasia of reticular tissue but not a more differentiated diagnosis

Cancer (*C*) This group included cases of carcinoma, liposarcoma, melanosarcoma, malignant glioma and myeloic leukemia, not assigned to any of the abovementioned diagnostic groups The diagnoses were in most cases confirmed by histological examination of biopsy and/or necropsy specimens The cases were divided into 2 subgroups according to whether bone marrow had been examined (C 1) or not (C 2)

Other diseases (*O*) This group included all other patients and was divided into two subgroups according to whether bone marrow had been examined (O 1) or not (O 2)

Results

Table I summarizes the immunologic types of the M-components and their concentration in the various diagnostic groups In cases with more than one M-component the serum concentration given is that of the total of the M components The concentration of the M components is not given for patients treated with cytostatics 2 weeks or earlier before collection of the samples The highest concentration of M-components of type γG was 10 3 g/100 ml, of type γA 8 6 g/100 ml and of type γM 8 1 g/100 ml A concentration above 8 0 g/100 ml was found in 13, 3 and 1 cases, with M components of type γG, γA and γM respectively

Immunologic classification demonstrated M components of type γG in 318 sera, of type γA in 109 sera, of type γM in 95 sera, and of type γ, (light chain proteins) in 18 sera Seven sera were found to contain both an M component of type γG and one of type

TABLE 1 Type and serum concentration of M components in relation to diagnosis Cyt treat = patients who had been treated with cytostatics 2 weeks or earlier before collection of the sample The concentration of the M-components is not given in these cases

M-component		Diagnosis								
Type	Conc g/100ml	M	A	L	N	C 1	C 2	O 1	O 2	Total
G	<1.0	8	6	6	–	16	3	53	24	116
	1.1–2.0	8	4	2	–	7	1	27	11	60
	2.1–3.0	15	10	2	–	–	–	5	–	32
	3.1–4.0	13	8	–	1	–	1	1	–	24
	4.1–5.0	14	2	2	–	–	–	2	–	20
	>5.1	44	2	2	–	–	–	2	–	50
	Cyt treat	*12*	*2*	*2*	–	–	–	–	–	16
	Total	114	34	16	1	23	5	90	35	318
A	<1.0	9	4	1	–	7	1	22	4	48
	1.1–2.0	11	1	1	–	–	–	–	–	13
	2.1–3.0	7	4	–	–	–	–	–	–	11
	3.1–4.0	9	–	–	–	–	–	–	–	9
	4.1–5.0	6	2	–	–	–	–	–	–	8
	>5.1	*8*	*1*	–	*1*	–	–	*1*	–	*11*
	Cyt treat	9	–	–	–	–	–	–	–	9
	Total	59	12	2	1	7	1	23	4	109
G–A	<1.0	–	–	1	–	2	–	–	–	3
	>1.1	4	–	–		–	–	–	–	4
	Total	4	–	1	–	2	–	–	–	7
M	<1.0	–	1	6	1	4	5	10	8	35
	1.1–2.0	–	–	11	–	2	–	11	2	26
	2.1–3.0	–	1	7	–	–	–	2	2	12
	3.1–4.0	–	–	2	1	–	–	–	1	4
	4.1–5.0	–	–	6	1	–	–	–	–	7
	>5.1	1	–	4	2	–	–	3	–	10
	Cyt. treat	–	–	1	–	–	–	–	–	1
	Total	1	2	37	5	6	5	26	13	95
D	<1.0	–	–	–	–	–	–	–	1	1
	1.1–2.0	1	–	–	–	–	–	–	–	1
	Cyt treat	1	–	–	–	–	–	–	–	1
	Total	2	–	–	–		–	–	1	3
κ	<1.0	11	2	–	–	–	–	1	–	14
	1.1–2.0	1	–	–	–	–	–	–	–	1
	Cyt. treat	2	–	1	–	–	–	–	–	3
	Total	14	2	1	–	–	–	1	–	18
λ p	<1.0	–	–	–	–	–	–	3	–	3
	1.1–2.0	–	–	–	–	–	–	1	–	1
	Total	–	–	–	–	–	–	4	–	4
	Total	194	48	57	7	38	11	144	53	554

γA In 3 sera an M component of a type other than those mentioned above was suspected These sera were examined by Dr Fahey (National Institutes of Health, Bethesda, U S A), who reported that they contained M-components of type γD In 4 sera the results obtained were atypical In some cases γ_μ-M components occurred together with other types of M-components, but the exact number cannot be stated

Diagnostic classification showed that the two commonest diagnoses were M and O, which together with A represented 79 % of the entire series Nine % of the cases were assigned to group C, and carcinoma had been demonstrated in a further 16 (3 %), namely in 7 with the diagnosis M, in 6 with A, in 2 with L, and 1 case with the diagnosis N The diagnosis of L was made in 57 cases (11 %) Among these were 9 cases with lymphatic leukemia with W B C above 20,000/mm³ and 24 cases who had the clinical diagnosis of macroglobulinemia Waldenstrom The diagnosis of M was made in 58 cases post mortem, in 70 cases on the basis of the bone marrow and roentgenographic findings, in 4 cases on the basis of bone marrow findings and of the skeleton changes according to the criteria for A, in 15 cases on the basis of skeletal roentgen findings and of the bone marrow findings according to the criteria for A, in 33 cases on the basis of bone marrow findings alone and in 14 cases on the basis of roentgenographic findings alone

Diagnosis in relation to type of M component In M the M-components were usually of type γG, γA or γ, and in L of type γM or γG

The diagnosis of N in one case with a γG-M component was based on sternal marrow examination which showed about 80 % small cells resembling both lymphocytes, plasma cells and fibrocytes, and in one case with a γA-M component on sternal marrow examination and biopsy of a rib tumour which showed a malignant tumour most like a reticulum cell sarcoma though myelomatosis could not be excluded The diagnosis of M in one case with a γ M M-component was based on examination of bone marrow from crista iliaca after sternal puncture had given no representative specimen In one case with a γA-M-component in a concentration of 5 3 g/100 ml the diagnosis was O The sternal marrow smear was poor in cells without definite pathological changes, the number of plasma cells was normal In 3 cases with γM M components in a concentration above 5 0 g/100ml the diagnosis was O In these cases sternal marrow examination and biopsy from crista iliaca in one case, and biopsy of an inguinal lymph node in another case, showed no specific changes The serum M-component was known in two of them for more than 10 years None of the above mentioned 7 cases had any roentgenologically demonstrable osteolytic lesions typical of myelomatosis

Compared with the frequency of M components in the entire material M-components of type γM were over-represented in patients with the diagnoses L and N and those of type γA under-represented in the diagnostic groups C and O

Two of the 3 patients with an M component of type γD had myeloma-

tosis, in both cases the diagnosis was based on bone marrow and skeleton X ray findings The third patient was not examined completely

Of the 7 cases with two M components, one of type γG and one of type γA, the diagnosis was myelomatosis in 4, carcinoma in 2 and reticulum cell sarcoma and renal carcinoma in 1 These cases have been described previously (3)

In the cases with M components of type γA the diagnosis was M in 14 (78 %) cases and A in 2 (11 %)

Diagnosis in relation to the serum concentration of M components of type γG The frequency of M increased with the concentration of the γG M components while in the majority of cases with a diagnosis of C or O the concentration of the M-components was 2 0 g/100 ml or less The diagnostic group A occupied an intermediate position with concentrations usually between 2 1 and 4 0 g/100 ml This is also clear from fig 2 (A) which shows the frequency of M and of M + A in relation to the concentration of the M-components The largest increase in frequency to 47 % for M and 78 % for M + A occurred when the concentration of the M-components rose from 1 1—2 0 g/100 ml to 2 1—3 0 g/100 ml

Diagnosis in relation to the serum concentration of M-components of type γM The number of cases with a diagnosis of M did not vary essentially with the concentration but in all cases except 3 in the other diagnostic groups the concentration was 1 0 g/100 ml or lower Fig 2 (B) shows that the frequency of M increased from 19 % to 85 % and that of M + A

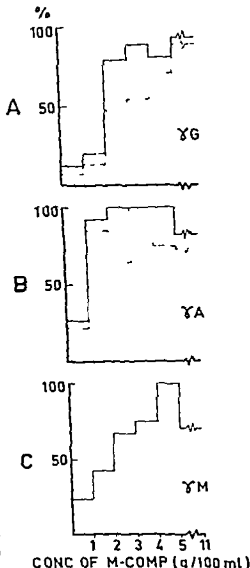


Fig 2 Frequency of myelomatosis (– – – –) and of myelomatosis – assumed myelomatosis (———) in relation to concentration of M components of type γG (A) and γA (B) and the frequency of clinically observed neoplasia of the reticular tissue (———) in relation to the concentration of M-components of type γM (C)

from 27 % to 92 % when the M-component concentration increased from 1 0 g/100 ml or less to 1 1—2 0 g/100 ml

Diagnosis in relation to the serum concentration of M components of type γM. The number of cases with the diagnosis of L was roughly the same irrespective of the concentration of the M components, while C and O were represented mostly among the cases with M component concentrations of 2.0 g/100 ml or less. The frequency of cases with neoplasia in the reticular tissue (L + M + A) therefore increased with the concentration of the M components (fig. 2 (C)). At concentrations above 2.0 g/100 ml this frequency increased to about 70 % or more.

Discussion

In the present material the diagnostic group O was larger (36 %) than corresponding diagnostic groups in the series of Waldenström (22), Osserman and Takatsuki (15) and Creyssel et al. (5), where it was 27 %, 10 % and 8 %, respectively. This difference may probably be explained by the fact that paper electrophoresis is now used on wider indications than formerly and that M components in low concentrations are not uncommon in a normal population above 50 years (2). This has substantially reduced the diagnostic value of M components per se. But when the concentration of the M components was considered, it was found that 78 % or more of the patients with a concentration of γG M components higher than 2.0 g/100 ml, and 82 % or more of those with a concentration of γA M components higher than 1.0 g/100 ml, had a definite or assumed diagnosis of myelomatosis (fig. 2 (A and B)), and that 67 % or more of the patients with a concentration of γM-M components higher than 2.0 g/100 ml had clinically observed neoplasia of the reticular tissue (fig. 2 (C)). The findings of a serum M component in a high concentration is thus still of considerable diagnostic value. This also holds for M-components of type γ_{μ} demonstrable by paper electrophoresis of serum, for myelomatosis was established or assumed in 89 % of the cases. In these cases the serum concentration of the M components was low, which can be explained by the rapid renal excretion of such M components.

The diagnostic group L included macroglobulinemia Waldenström, lymphatic leukemia, lymphosarcoma, reticulum cell sarcoma and related conditions. In some patients it was difficult to distinguish between these conditions, partly because the material consisted of patients from various hospitals using a somewhat different diagnostic classification. Moreover there is a close relationship between these conditions, and transitions from one to the other have been described (22, 24). Neither did Osserman and Takatsuki (15) differentiate between different malignant lymphomas without taking the type of M component into consideration in the presentation of their material.

The difference in frequency of myelomatosis in relation to the serum concentration of M components of type γG and γA is remarkable. In patients with γA M components the frequency rose considerably at a lower concentration than in patients with γG M components (fig. 2 (A and B)). This

might indicate a more rapid fractional rate of catabolism of M components of type γA than of M components of type γG, if it is assumed that the production rate of M-components is of about the same magnitude in myelomatosis irrespective of the type of the M component. So far the turnover of γA M components has been studied in only a few cases. Gabuzda (9), who used an isotope technique, found no difference in T 1/2 between a case with a γA M component and cases with γG M components, while Alper et al. (1) and Drivsholm (6), in one and three cases respectively with γA M components found a lower T 1/2 than in most cases with γG M components. It might also partly explain why the frequency of the diagnostic groups C and O in our material was relatively lower in cases with M components of type γA than in those with M components of type γG. A higher rate of production of γA globulins would then be necessary for them to be demonstrable as M components. If also the normal fractional rate of catabolism of the γA globulins is higher than that of the γG globulins, it could partly explain why we like others (5, 7, 15, 23) found M components of type γG to be roughly twice as common as those of γA in myelomatosis, while the ratio between the normal serum concentration of γG and γA globulins is about 5:1 (8, 10).

In the previously published case with an M-component of type γD (17) the patient had myelomatosis, a diagnosis made also in 2 of our 3 cases, while the third was not completely examined. It therefore seems reasonable to assume that the clinical significance of M components of type γD is comparable to that of types γG and γA.

Patients with M components without signs of myelomatosis or other neoplasms of the reticular tissue have often been found to have carcinoma or some other neoplasm (1, 14—16, 22, 23). They have therefore been assigned to a separate diagnostic group. The frequency of M components in low concentration has however been found to be 3 % in the aged (11) and as high as 1—6 % in the ages 50—90 years in a normal population (2), so that there need not be any important relation between M components and carcinoma. In our material no difference was found between the diagnostic groups C and O regarding the distribution of M components according to type or concentration.

Summary

In 554 cases with serum M components the clinical diagnosis was related to the type and concentration of the M components. 318 cases had M components of type γG, 109 of type γA, 95 of type γM, 3 of type γD and 18 of type γ_μ. Seven cases had one M component of type γG and one of type γA. In 4 cases the results obtained were atypical.

Clinically manifest myelomatosis was seen in 194, assumed myelomatosis in 50, malignant lymphoma including macroglobulinemia Waldenström, lymphatic leukemia, lymphosarcoma and related conditions in 57, undifferentiated neoplasia of the reticular tissue in 7, other cancers in 49 and other conditions in 197 cases.

The frequency of clinically manifest and assumed myelomatosis increased with the serum concentration of M-components of type γG and γA. In patients with γG-M components it increased from 12 % at a concentration of 1.0 g/100 ml or less to 78 % or more at a concentration above 2.0 g/100 ml. In patients with γA-M components it increased from 27 % at a concentration of 1.0 g/100 ml or less to 82 % or more at a concentration above 1.0 g/100 ml.

The frequency of neoplasia of the reticular tissue (macroglobulinemia Waldenstrom, lymphatic leukemia, lymphosarcoma, reticulum cell sarcoma and allied conditions) increased with the concentration of M components of type γM from 23 % at a concentration of 1.0 g/100 ml or less to 67 % or more at a concentration above 2.0 g/100 ml.

Two patients with M-components of type γD had clinically manifest myelomatosis. In 16 of 18 cases with serum M component of type γ_μ the patients had clinically manifest or assumed myelomatosis.

Acknowledgement

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SUBJECT INDEX

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The frequency of neoplasia of the reticular tissue (macroglobulinemia Waldenstrom, lymphatic leukemia, lymphosarcoma, reticulum cell sarcoma and allied conditions) increased with the concentration of M components of type γM from 23 % at a concentration of 1 0 g/100 ml or less to 67 % or more at a concentration above 2 0 g/100 ml

Two patients with M components of type γD had clinically manifest myelomatosis In 16 of 18 cases with serum M-component of type γ_μ the patients had clinically manifest or assumed myelomatosis

Acknowledgement

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